

RESEARCH HIGHLIGHTS
NATIONAL INSTITUTES OF HEALTH

1959

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
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Items of Interest on
Program Developments and Research Studies
Conducted and Supported by the Institutes
and Divisions of NIH

As Presented to the
Congress of the United States

INTRODUCTION

For several years the National Institutes of Health, main research center of the Public Health Service, has submitted statements supplementing its testimony at congressional hearings on appropriations to the U. S. Department of Health, Education, and Welfare. The following pages comprise the statements called Research Highlights as prepared by the Institutes and Divisions of NIH for hearings on the fiscal 1961 budget.

The compilation is intended to meet requests and administrative needs. It contains representative items selected in light of both scientific importance and public interest. In no sense is it a comprehensive review of NIH activities, nor even necessarily of its most significant results.

The items concern the work of both staff scientists (laboratory and clinical) and research grantees. No attempt has been made to reflect the relative magnitude of the grants program. During the period covered--calendar year 1959--more than three-quarters of NIH funds for research were awarded to investigators in universities, medical schools, and other non-Federal research centers. 195

The items in this report are based on published work. The scientists' articles appear in a wide range of journals, mainly non-Federal, which are available in medical libraries. Further information, including references to these articles, may be obtained through the Office of Research Information, NIH, or the information office of the Institute concerned.

CONTENTS

Introduction	1
National Cancer Institute	3
National Heart Institute	49
National Institute of Allergy and Infectious Diseases	109
National Institute of Arthritis and Metabolic Diseases	167
National Institute of Dental Research	203
National Institute of Mental Health	225
National Institute of Neurological Diseases and Blindness	277
Clinical Center	331
Division of Biologics Standards	337
Division of General Medical Sciences	351
Division of Research Services	377

Office of Research Information
National Institutes of Health
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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

HIGHLIGHTS OF PROGRESS

IN CANCER RESEARCH

1959

Items of Interest on Research Studies Conducted and Supported by the National Cancer Institute

The highlights of accomplishment in the many scientific disciplines that are focused on the problem of malignant disease result from, and at the same time contribute to, the changing pattern of cancer research. In the past year, special emphasis was given to the development of programs designed to find out whether human cancer is a viral disease, devise procedures for detecting cancer in its earliest stages, and produce drugs for more effective treatment. Concurrently, basic research continued to provide information on the biological and biochemical nature of living matter, on differences between the normal and cancerous states, on host-tumor relationships, and on the response of cells and organisms to the damaging effects of radiation, chemicals, and other agents.

In 1959, the isolation of a mouse leukemia virus from a solid tumor was reported. Rats that were not previously made susceptible to transplants of foreign tissue by X-irradiation or cortisone supported the growth of human cancer tissue when they were inoculated with the tissue a few days before birth. A new drug related to nitrogen mustard given once a week to leukemic mice produced a 200 percent increase in median survival beyond that of untreated control animals. A hormonal drug related to testosterone was somewhat more effective in the palliative treatment of advanced breast cancer than the standard therapeutic agent from which it was derived.

The items presented in this report are representative of the highlights of program developments and progress in research conducted and supported by the National Cancer Institute. Institute staff scientists are identified by the Laboratory or Branch with which they are connected and grantees by their institution. Most of the results were reported by groups of investigators, but for the sake of brevity; only the senior author is identified in the following summaries.

VIRUS STUDIES

NEW MOUSE LEUKEMIA
VIRUS IS EXTRACTED
FROM SOLID TUMOR

A virus that produces leukemia in mice has been isolated from transplantable sarcoma 37 by Dr. John B. Moloney, of the

Laboratory of Biology. The first leukemias resulting from injection of cell-free materials from sarcoma 37 were seen during September 1958, among mice that had received the material shortly after birth some eight months earlier. The activity of the agent has been greatly increased so that it now produces leukemia within 10 weeks in all mice injected on the first day of life.

In contrast to other leukemogenic viruses affecting mice, Dr. Moloney's agent elicits the disease in several different strains, is active in adults as well as newborns, and produces a leukemia indistinguishable from the type occurring commonly as a spontaneous disease in this species. The relationship of the newly discovered viruses to other viruses of the mouse leukemia group remains to be defined, and immunological studies are in progress to achieve that objective.

Dr. A. J. Dalton, also of the Laboratory of Biology, has seen bodies that probably represent the virus under the electron microscope in centrifuged pellets and in leukemic cells. They have an average diameter of about 85 millimicrons and possess a characteristic internal structure.

VIRUS CAUSING MULTIPLE
TUMORS IN MICE SHOWN
TO BE SINGLE ORGANISM

Dr. Sarah E. Stewart, Laboratory of Biology, and Dr. Bernice E. Eddy, Laboratory of Viral Products, Division of Biologics Standards,

have reported extensively on their collaborative studies of an agent, SE polyoma virus, first obtained from the tissues of leukemic mice. The study originated in observations by Dr. Stewart that injection of newborn mice with cell-free mouse leukemia extracts produced an unusual type of tumor of the parotid (salivary) gland that rarely occurs spontaneously in mice. (See Highlights of Progress in Research on Cancer, 1958, pp. 1-2),

Dr. Stewart and her coworkers undertook to determine whether the polyoma virus is a single organism or a group of agents each of which causes malignancy in a specific tissue. A 1 million-fold dilution of cell-free fluid from tissue cultures of the virus was poured over a flat surface of mouse embryo cells in a Petri dish. By this procedure, individual virus particles were distributed about the surface. As the particles multiplied, they damaged the cells, producing plaques presumably

representing colonies derived from a single virus particle. Each plaque was inoculated into tissue culture, and the tissue culture fluids were subsequently injected into newborn mice.

Nearly 90 percent of the mice injected developed tumors. Salivary-gland tumors were found in 96 percent of the mice that developed neoplasms; lesions of the kidneys in 50 percent; and tumors of the thymus, mammary glands, and bone in 25 percent. Other malignancies included tumors of the hair follicles, thyroid, and adrenal glands.

Because the same spectrum of tumors developed in the mice, following inoculation of plaqued virus and of original starting material, it was concluded that only one virus was present in all the plaques tested.

(Drs. Eddy and Stewart have now reported that the polyoma virus, which causes multiple tumors in mice and also neoplasms in hamsters, produces neoplasms in rats as well, when introduced into new-born animals. Other studies reported by Drs. Eddy and Stewart have shown that immunization of female hamsters during pregnancy substantially reduces the incidence of tumors in offspring inoculated at birth with polyoma virus. Details of these studies are given in Highlights of Progress in Research on Biologics, 1959).

SE POLYOMA VIRUS IS SEEN UNDER ELECTRON MICROSCOPE

Other aspects of research on the polyoma virus are being explored by a number of investigators. Dr. Herbert Kahler, Laboratory of Physiology, and scientists of the National Institute of Allergy and Infectious Diseases, have reported on electron microscope studies of the virus.

Crude tissue-culture fluid from a culture of mouse parotid-gland tumor and a culture of mouse mesenteric lymph-node tumor was concentrated and purified in order to prepare the material for study under the electron microscope.

Electron photomicrographs showed a characteristic particle, which was seen as a slightly flattened oblate spheroid with a diameter of 59 mu in isolated particles and as spheres with a diameter of 40 to 45 mu in groups of particles. Calculation of a more characteristic constant for the desiccated particle when in spherical form gave a value of 44 mu. This size is somewhat smaller than that of the rabbit papilloma virus.

Electron microscope studies of the polyoma virus grown in cell cultures of a mouse lymphoma have been reported by Dr. William G. Banfield, Laboratory of Pathology. Spherical particles were found in the nucleus, cytoplasm, and on the surface of the cell in infected cultures. Newborn mice inoculated with these cultures developed the salivary-gland tumors and multiple tumors of other types characteristically associated with the polyoma virus. Control cultures did not contain the particles, nor did mice receiving control cultures develop tumors.

The average diameter of the particles was 27 to 35 mu, a size comparable with that obtained in the studies reported by Dr. Kahler.

VIRUS SEEN IN MOUSE
TUMORS THAT RESEMBLE
HUMAN MULTIPLE MYELOMA

Scientists of the General Medicine Branch reported about two years ago that several mouse plasma cell tumors are strikingly similar to human multiple myeloma in microscopic appearance of tissue, development of bone lesions, and production of an abnormal blood serum protein. Investigation of the mouse tumors was undertaken in an effort to acquire knowledge that might be applicable to the human disease.

Dr. Albert J. Dalton, Laboratory of Biology, studied the fine structure of six plasma cell tumors under the electron microscope to determine whether they have some common morphologic feature that would account for their similarity to one another.

He reported that a significant characteristic common to all six plasma cell tumors examined was the presence of virus-like particles in various stages of formation within the tumor cells. There is no indication that the virus particles are associated with the etiology of the mouse tumors, however.

Further studies will include efforts to determine whether viruses are involved in causation of the mouse plasma cell neoplasm and to extend the results to investigations of human multiple myeloma.

INCREASED EMPHASIS PLACED
ON INVESTIGATION OF HUMAN
VIRUS-CANCER RELATIONSHIP

In the past year, increased emphasis was placed on research to find out whether viruses cause human cancer. The leading problem is to devise means for determining whether a virus isolated from human tumor tissue caused the disease. Such tests cannot be conducted in man; therefore, laboratory

procedures must be developed.

The National Cancer Institute awarded 48 new grants for long-term support of intensive research in this area to scientists in the United States and abroad. Several of the grantees are eminent virologists who have made significant contributions in fields other than cancer. The awards, totaling a little less than \$ 2 million for the first year, brought the number of the Institute's current virus grants to 110, and the allocation for virus-cancer grants to about \$3.9 million, about twice the amount previously awarded for such work.

Much of the work being done under these grants involves studies of virus-caused tumors in laboratory and domestic animals. These investigations are chiefly intended to provide scientific information applicable to the study of the human cancer-virus relationship. Efforts to identify viruses that may have produced human cancer are facilitated by the use of highly refined laboratory tools such as the electron microscope, and techniques such as tissue culture.

One result of the increased interest in virus-cancer research was a conference, "The Possible Role of Viruses in Cancer," sponsored by the American Cancer Society and held in Rye, New York, in November. The scientists attending the meeting represented a number of important fields, such as virology, tumor virology, biochemistry, genetics, and tissue culture. Many of the participants were Institute grantees. Discussion centered around the present state of knowledge of viruses and cancer, the present aims of virus-cancer research, and possible means of achieving them. It was emphasized that there is a great need to characterize the available test systems and to develop new ones. There is also need for adequate sources of living host and virus materials for use in research.

ENVIRONMENTAL CARCINOGENS

GRANTEES REVIEW DATA ON AIR POLLUTION AND LUNG CANCER CAUSATION

In the opinion of many investigators, multiple factors are operative in the production of lung cancer. Scientists of the

University of Southern California School of Medicine reviewed a large amount of experimental data on the role and mechanism of action of air pollutants in the pathogenesis of lung cancer and Dr. Paul Kotin has summarized representative chemical, physical, and biological findings.

A number of substances that may be carcinogenic have been identified as atmospheric pollutants. These include: 3,4-benzpyrene, oxidation products of aliphatic hydrocarbons present in gasoline vapors, and specific inorganic materials such as chromium and nickel compounds. The compound, 3,4-benzpyrene, is one of the most widespread of these agents; it has been isolated from gasoline and diesel engine exhausts, soots produced in the incomplete combustion of organic matter, and in some areas several specific industrial effluents. Several other aromatic polycyclic hydrocarbons not previously characterized have been identified in the atmosphere and are now under bioassay for carcinogenicity.

The soot particles on which carcinogenic hydrocarbons are adsorbed are in a size range in which they can be deposited and retained when inhaled. Respiratory epithelial changes occur which facilitate the deposition and retention of such particles. Removal of the carcinogen from the soot by body fluids is suggested by the finding that soot obtained at autopsy from human lungs has been shown to be free of the carcinogen, 3,4-benzpyrene.

Dr. Kotin concludes with the suggestion that the development of lung cancer is the end stage of a series of sequential changes that require the presence of a carcinogenic agent, environmental host-modifying factors, and the innate susceptibility of the host.

**LOWER BURNING TEMPERATURE
OF CIGARETTE TOBACCO MAY
LESSEN LUNG CANCER RISK**

Numerous scientific observations have indicated that high molecular weight polycyclic hydrocarbons can be formed by pyrolysis

(combustion at high temperatures) of relatively simple aliphatic hydrocarbons. Hence, it has been suggested that the carcinogenic activity of tobacco smoke condensate is a consequence of the pyrolysis of tobacco. Dr. Ernest L. Wynder and his colleagues at the Sloan-Kettering Institute for Cancer Research, New York, and the University of Toronto have now reported the results of studies of the pyrolysis of tobacco extracts.

Tobacco extracts consisting essentially of paraffinic and polyenic hydrocarbons were pyrolyzed at temperatures ranging from 880° C., the approximate combustion temperature of cigarette tobacco during smoking, to 560° C. The resulting materials (pyrolysates) were dissolved in acetone and applied repeatedly to the backs of Swiss mice and the ears of New Zealand rabbits.

The tumorigenic activity in mice of the 880° C. pyrolysate was greater than that of any other material studies, as

reflected by a short latent period of tumor formation and the development of tumors in all animals surviving three months. Activity decreased with decreasing pyrolysis temperature, from slightly lower activity with the 800° C. pyrolysate to no activity with the 560° C. material. Results obtained in rabbits were comparable with those obtained in mice.

In other experiments, it was found that the manner of smoking cigarettes; i.e., producing different puff volumes or smoking different lengths of cigarettes did not alter the carcinogenic activity of tobacco tar on a gram per gram basis.

Dr. Wynder concludes from these investigations that temperature plays a critical role in the formation of carcinogenic hydrocarbons in cigarette tar and suggests that lowering the combustion temperature to the range of 700° C. would provide a lead to a practical solution to the tobacco-cancer problem.

METABOLITES OF SUBSTANCE CAUSING BLADDER CANCER IN MAN STUDIED IN DOGS

Other studies of environmental carcinogens are designed to produce information on the process by which these substances

cause cancer.

A few aromatic amines, such as beta naphthylamine, 4-aminodiphenyl, and benzidine have been associated with bladder cancer among workers in the dye industry. Administration of the first two substances in pure form produces bladder cancer in dogs. However, only a few dogs have ever developed bladder cancer after benzidine exposure. This situation prompted investigators at the Yale University School of Medicine to study the fate of benzidine following injection into dogs.

Benzidine was administered intraperitoneally. The urine of the dogs was collected for chemical analysis; urine collected for at least one day before benzidine injection furnished control values for the analyses.

The results reported by Dr. Louis J. Sciarini showed that about half the injected dose of benzidine was excreted in the urine as the metabolite (breakdown product) 3-monohydroxybenzidine. Free benzidine recovered in the urine comprised about 7 percent of the administered dose. Although studies of the fate of benzidine in man have produced only small amounts of free benzidine and metabolites, the major metabolite in man is identical with that in the dog. However, in addition, there is good evidence that acetylated benzidines are present

in human urine; these metabolites were not detected in the present studies on dogs.

Dr. Sciarini suggests that the benzidine metabolites discovered to date may not be the actual carcinogens responsible for the production of bladder cancer. Further studies on the biotransformation of benzidine to 3-mono-hydroxybenzidine in other laboratory animals and in human workers and biological tests of the major urinary metabolites will provide additional information on the carcinogenic action of benzidine.

TUMORS IN LABORATORY ANIMALS

DESCRIBE TUMORS IN NEW AGENT-FREE MOUSE STRAIN

In studies of mammary tumors there is often a need for a strain of mice that does not have the mammary tumor agent but is genetically susceptible to it. One such strain on which extensive observations have been made by National Cancer Institute scientists is C3Hf.

Dr. Margaret K. Deringer, Laboratory of Biology, National Cancer Institute, has now reported the development of a new strain of agent-free mice and described the occurrence of tumors in these animals. This new strain, designated C3He, was produced by transfer of fertilized ova from agent-carrying, high-tumor C3H mice to the uteri of agent-free, low-tumor C57 BL animals. Offspring of this transfer were nursed by their foster mothers. The new colony was established by mating a pair of these foster-nursed animals.

Mammary tumors developed in 4 percent of the virgin females at an average age of 20.7 months, 54.8 percent of breeding females at 19.2 months, 74.4 percent of the force-bred females at 17.9 months, and 22.4 percent of males treated with the estrogen, diethylstilbestrol, at an average age of 15 months. These figures were comparable with the results obtained with the C3Hf mice, except for a greater incidence of mammary tumors in breeding females of the C3He substrain. As was observed in the C3Hf strain, the more litters a female produced the greater was the incidence of mammary tumors in the C3He strain. (Highlights of Progress in Research on Cancer, 1958, p. 4).

One outstanding observation of this study was the high frequency of hepatomas in the C3He mice. They developed in 58.6 percent of virgin females at 24 months, 30.3 percent of breeding females at 21 months, 37.8 percent of force-bred

females at 20 months, 55.1 percent of treated males at 23 months, and in 90.5 percent of breeding males at 21 months. This is the highest occurrence of spontaneous hepatomas ever reported for any strain of mice.

Ovarian tumors were observed in 47.5 percent of virgin females, 37.4 percent of breeding females, and 29.3 percent of force-bred females. The differences among the three groups were attributed to the fact that the virgins lived longer and more of the force-bred females died early with mammary tumors.

The present study appears to confirm previous indications that genetic susceptibility, hormonal stimulation from breeding, and advanced age of females are all factors responsible for the appearance of tumors in agent-free strains of mice.

CHARACTERISTICS OF TUMOR APPEAR RELATED TO EXCESS ADRENAL HORMONE SECRETION

Scientists of the Laboratory
of Pathology observed the
appearance of a spontaneous
tumor of the adrenal cortex

in a group of National Institutes of Health Osborne-Mendel rats. This malignancy, designated Adrenal cortical carcinoma 494, and two subline tumors derived from it has provided the basis for a study aimed at yielding information on the effect of hormone balance on tumor growth.

Dr. Katharine C. Snell has reported that tumor 494 was maintained through 7 transplant generations in male and female Osborne-Mendel rats. About 10 weeks after transplant, all animals developed excessive thirst and urine passage. At autopsy, they presented degenerative changes of kidney tubules, and atrophy of adrenal and pituitary glands, reproductive organs, and lymphoid tissue. Histologic examination showed that cells of the tumor were arranged in cords and were nearly all polygonal and of uniform size.

In a subsequent experiment, tumor 494 was injected into rats from which the pituitary gland had been removed. The resultant tumors differed from line 494 in that they consisted of large, bizarre cells in addition to the uniform cells of the parent tumor. When reintroduced into adult, intact rats, the subline tumor retained its histologic pattern, but like the original tumor, induced excessive thirst and urination. Moreover, the subline tumor induced hyperplastic mammary glands filled with milky fluid in both males and females, atrophy of male sex organs, and changes in female sex organs characteristically seen following administration of estrogens.

A second subline was produced by injecting tumor 494 into sexually immature rats. This subline was histologically similar to that produced in rats lacking pituitary glands, and it induced similar physiological changes,

The results suggest that carcinoma 494 and the two sublines secreted an excess of certain adrenal cortical hormones, and that these secretions, by suppressing the pituitary gland, indirectly induced atrophy of the adrenal glands, sex organs, and lymphoid tissue. Damage to the kidneys and the resultant elevation of thirst and urine passage may have been caused directly by secretions from the tumor acting on the kidney. The differences between tumor 494 and its two sublines cannot be explained at present, but Dr. Snell suggests that altered histologic appearance and physiological activity of the subline tumors may result from the lack of a restraining influence in animals from which the pituitary has been removed or sexually immature animals.

HUMAN CANCER NOW
GROWN IN UNTREATED
LABORATORY ANIMALS

Growing human cancer in
laboratory animals will enable
scientists to study ways of
detecting, treating, and

possibly curing human malignancies, and may be of importance in studying the possible viral etiology of human cancer. Previous investigations have shown that human tumors will grow in animals treated with X-irradiation and cortisone. Dr. Fred W. Gallagher, University of Vermont, has now reported a method for growing cancer in untreated laboratory animals. This was accomplished by inoculating rats before birth.

The stock tumor was human cancer H Ep 3, which was obtained from the Sloan-Kettering Institute for Cancer Research and maintained by serial passage in X-irradiated, cortisone-treated weanling Wistar rats. In this study, malignant tissue from the stock animals was suspended in salt solution and inoculated into the thoracic cavity, peritoneal cavity, or subcutaneous tissue of rat fetuses.

Most of the animals were born alive and a high percent had cancers. The tumors grew rapidly, and those in the thoracic and peritoneal cavities killed more quickly and regularly than did the subcutaneous growths. Some of the subcutaneous tumors began regressing within two weeks after inoculation and finally disappeared.

Most of the animals inoculated at 19-20 days gestation age developed cancers. Fetuses injected just a few hours before birth (22nd day of gestation) did not show as many tumors as those injected earlier.

The tumors from these animals were passed in series in new litters of unborn rats and also produced tumors in X-irradiated, cortisone-treated weanling rats. In both instances, the tumors retained their original characteristics.

The results suggest that successful proliferation of H Ep 3 human cancer in untreated laboratory animals was achieved by taking advantage of the increased susceptibility of the fetus, so that the tumors were established before the development of the rats' natural resistance at birth.

NEW METHOD OF PRODUCING MOUSE TUMOR RESEMBLING HUMAN CANCER IS REPORTED

One of the striking characteristics of human multiple myeloma is the frequent production of

large amounts of abnormal plasma proteins. Since plasma-cell tumors also produce these abnormal plasma proteins in animals, a better understanding of multiple myeloma may be possible through studying experimental plasma-cell neoplasms.

Dr. Ruth M. Merwin, Laboratory of Biology, has reported the unexpected occurrence of plasma-cell tumors in BALB/c mice in an experiment originally designed to study passage of the mouse mammary tumor agent through diffusion chamber membranes. These induced plasma-cell tumors are of particular interest because they are transplantable--four have been maintained in two or more transplant generations--and they synthesize abnormal proteins.

Microscopic examination of the plasma-cell neoplasms revealed large cells with plasma-cell characteristics. Metastases were found in the ovary, pituitary gland, spleen, and lymph nodes. One tumor line of the plasma-cell tumor produced a myeloma-type change in the kidney, while another caused osteolytic lesions.

UPTAKE OF RADIOACTIVE AMINO ACIDS STUDIES IN TUMOR-BEARING ANIMALS

Information on the relationship between nutrition of a tumor and that of its host is important to an understanding of

the uncontrolled growth of tumors. One approach to the problem is the study of the supply of nutrients and their pattern of distribution and utilization within a tumor.

In a study of dynamic factors involved in the growth of tumors, scientists of the Laboratory of Physiology have made an autoradiographic study of the uptake of radioactive amino acids in tumor-bearing rats and mice. The experimental procedure consisted essentially of administering a single

dose of radioactive amino acid to a tumor-bearing host, subsequently killing the animal, slicing the tumor in half, and preparing an autoradiogram from the cut face. Two rat tumors and two mouse tumors were investigated with glycine- $1-C^{14}$ and L-lysine- $U-C^{14}$.

Dr. James C. Reid reported that similar results were obtained with all combinations. The amino acids were taken up uniformly across the viable tissue; necrotic tissue did not take up amino acids; a viable area that had taken up an amino acid and then become necrotic released the amino acid only very slowly.

The observations as a whole suggest that tumor proteins are not metabolically inert, that the supply of nutrients is essentially diffuse, and that protein synthesis and presumably growth occur at a uniform rate throughout the viable tumor mass.

STUDIES AT THE CELLULAR LEVEL

GROWTH OF HUMAN CELLS IN SERUM-FREE CULTURE MAY AID VIRUS STUDIES

The search for viruses in human neoplasms may be aided by studying cell lines grown in serum-free media, since

even small amounts of animal or human serum may contain virus inhibitors. Dr. Alan S. Rabson, and Frances Y. Legallais, Laboratory of Pathology, with Dr. Samuel Baron, Laboratory of Viral Products, Division of Biologics Standards, previously reported the adaptation of a strain of animal tumor cells in a culture medium in which sterilized nonfat milk was substituted for serum. Now, in collaboration with Frances Y. Legallais, he has reported that a strain of cells from a human epidermoid carcinoma grew equally well in this medium.

In this study, malignant tissue obtained from the biopsy specimen of a facial skin lesion was cultured in a serum-free medium containing 20 percent autoclaved nonfat milk. The cell strain has been maintained for more than 2 years and carried through eight consecutive subcultures. Its structural characteristics of epidermoid differentiation have been preserved during this interval.

STUDIES ON HIGH AND LOW TUMOR-PRODUCING CELL LINES CONTINUE

Previous reports by scientists of the Laboratory of Biology have described results of studies of two cell lines grown

in tissue culture from a single mouse cell. These cell lines possess marked differences in morphologic, metabolic, and

growth characteristics. When implanted into mice of the inbred strain of origin, cells from the "high" line grow into malignant tumors in 97 percent of the mice; cells from the "low" line form tumors in only 1 percent. (Highlights of Progress in Research on Cancer, 1958, p. 18).

Dr. Katherine K. Sanford has now reported that studies of pure strains of cells (clones) each grown from a single cell, have led to the conclusion that the two lines do not differ in the proportion of malignant, transplantable cells that they contain, but rather possess entirely different heritable characteristics. This accounts for the low incidence of "takes" and the prolonged latent period for tumor development in vivo shown by the "low" line. A coauthor of the report was Dr. James M. Young, of the National Naval Medical Center, Bethesda.

Results of investigations of the metabolic properties of these clones are summarized in another report by Dr. Mark W. Woods, Laboratory of Biochemistry, who collaborated with the Laboratory of Biology investigators. The "high" line and the clones derived from it utilized approximately three times as much glucose as the "low" line and its clones. The clones derived from the "low" lines were less uniform; one showed glycolytic activity intermediate between that of the "high" and "low" line cells and thus appeared to be an intermediate in the progression toward an increased glycolytic capacity in vitro.

METASTATIC AND PRIMARY HUMAN TUMOR CELLS DIFFER IN CHROMOSOME COMPOSITION

Repeated observations have shown that the characteristic diploid number of chromosomes of experimental tumors may

change after many transplant generations. This change is always toward a higher value of ploidy, an increase in chromosomal number.

In an attempt to obtain information about changes in ploidy in human cancer, Dr. Giancarlo Rabotti, a visiting scientist in the Laboratory of Pathology, compared the chromosomal composition of cells in human primary tumors with that of their metastases. The amount of deoxyribonucleic acid (DNA) per nucleus, which provides information on the chromosome count, was determined by a microspectrophotometric technique.

The results showed that in all instances the diploid (2DNA) chromosomal arrangement characteristic of normal cells was evident in the control lymphocytes and primary tumor cell nuclei. In addition, some degree of hetero-polyploidy (4 DNA and 8 DNA)

was found in the primary tumor. On the other hand, the metastatic lesions contained fewer diploid nuclei and higher proportions of abnormal or heteropolyploid forms.

Dr. Rabotti concluded that there may be two explanations for the differences in chromosome ploidy between primary tumors and metastases: While all types of cells may be discharged from the primary tumor into the blood or lymph, only the heteroploid cells may be able to establish metastatic foci. Or, only heteroploid cells may leave the tumor to establish secondary growths.

PROGRESS IS REPORTED
IN STUDY OF TUMOR CELLS
IN CIRCULATING BLOOD

A progress report in the continuing study of the significance of tumor cells in the circulating peripheral

blood has been made by Dr. J. C. Pruitt, and his associates of the Field Investigations and Demonstrations Branch.

Dr. Pruitt has reported that a total of 300 blood specimens was examined by the quantitative technique recently developed at the National Cancer Institute. By this method human whole blood is processed so that it can be examined cytologically for the presence of malignant cells. The specimens were obtained from 100 patients with a diagnosis of cancer and from 200 presumably well people with no evidence of cancer and no history of the disease.

Malignant cells were identified in 39 percent of cancer patients and suspicious cells were present in an additional 12 percent. In the control group of 200 persons, on the other hand, cells that were considered to be malignant were found in only 1 person, or 0.5 percent.

An important result established by the study is the high percentage of cancer patients who have malignant cells that appear to be viable circulating in the blood stream.

SERUM CONTAINING
ANTIBODY INACTIVATES
RAT TUMOR CELLS

Investigators are increasingly concerned with the problem of resistance to cancer. In order to obtain information that may

eventually be applicable in human cancer, intensive studies are being made of immune mechanisms in laboratory animals.

Scientists at The Wistar Institute, Philadelphia, and the School of Medicine, University of Pennsylvania, have studied the immunity produced in rats against a transplantable ascites tumor. This tumor arose spontaneously in a rat of the inbred P.A. strain. It is always lethal when inoculated

intraperitoneally, but fails to grow when inoculated subcutaneously. Rats were immunized by subcutaneous inoculation of 0.05 ml of the ascites tumor, and the immune serum was obtained by cardiac puncture.

Dr. Paul M. Aptekman reported that there is a cytotoxic antibody in the immune serum, capable of inactivating tumor cells both in vitro and in vivo. Furthermore, the immune serum affords protection when administered to rats prophylactically (before inoculation of tumor) or therapeutically (after inoculation of tumor). The neutralizing ratio (number of tumor cells inactivated by 1 ml of immune serum) was 2,000,000 measured in vitro; 400,000 in vivo prophylactically; and 1,800,000 in vivo therapeutically. When the number of tumor cells rose to 2,000,000, therapeutic administration of serum produced only an extension in survival time. It was of interest to note that there was a critical and consistent ratio of tumor cells to immune serum, which protected in vivo, regardless of the total number of tumor cells present.

BIOCHEMICAL STUDIES

ELECTRON MICROSCOPE USED TO STUDY CELL COMPONENTS

One of the most important constituents of the cell is nucleic acid, which is thought

to carry the hereditary characteristics of an organism. One nucleic acid, DNA, is of particular interest to the cancer investigator because of the possibility that it is involved in the chemotherapy of cancer, and also in the process that converts a normal cell into a malignant one.

Many studies have been made of the chemical nature and structure of DNA, and a concept has been developed in the last few years of DNA as a helix of two intertwined chains of nucleotides. Now, Dr. Cecil E. Hall has reported on electron microscope studies of some polynucleotides, presumed to be DNA precursor substances. The work was done at the Massachusetts Institute of Technology, Cambridge.

The polynucleotides had molecular weights of 100,000 to 1,000,000. They appeared in the electron micrographs in general as well-defined rods in single-, double-, and triple-stranded helices.

Dr. Hall concludes that the electron microscope method is well suited to providing visual information on individual polynucleotides. The electron micrographs are of value in demonstrating distribution of lengths of molecules and such morphologic features as smoothness and state of aggregation.

STUDY NATURE OF AGENT
THAT CHANGES VIRUSES

Dr. Joseph Shack, Laboratory
of Physiology, has been
collaborating with Dr. Lawrence

Kilham, Division of Biologics Standards, in a study of the agent that transforms fibroma virus into myxoma virus in tissue culture. In view of the current interest in the role of viruses in cancer, these investigators are attempting to characterize the myxoma virus transforming agent (TAM) and, in particular, to determine the nature of the nucleic acid involved.

Earlier findings showed that TAM is essentially a myxoma virus particle that has lost infectivity due to denaturation of its outer protein coat. TAM was prepared by heating myxoma virus at 65° C. The study now reported was designed to find a way of removing the protein coat and exposing a postulated inner core of DNA without loss of transforming activity.

Treatment with urea resulted in no apparent reduction in transforming ability of heat-treated TAM, and rendered TAM susceptible to the destructive action of the enzyme, deoxyribonuclease (DNase). DNase had no effect on TAM prepared by heat alone.

It is not yet possible to decide whether the particles of UREA-TAM are without a protein coat or contain crevices which permit approach of the enzyme to the nucleic acid. Additional studies are being made to elucidate the nature of UREA-TAM.

NEW TECHNIQUE USEFUL
IN SEPARATING COMPLEX
BIOLOGICAL MATERIALS

Dr. Herbert A. Sober and
Elbert A. Peterson, Laboratory
of Biochemistry, have reported
the development of a **chromato-**

graphic technique for the separation of macromolecules of biological origin. The technique involves the adsorption of the mixture to be analyzed on a column of ion exchange cellulose. Removal of the individual components is accomplished by passage of suitable solvents through the column.

The investigators have now published a report summarizing some of their work and reviewing related findings of other investigators. By variation of the gradient of solvent, it has been practicable to obtain proteins in undenatured form with a facility and resolution heretofore impossible. For example, a chromatogram of normal serum eluted by a gradient selected to produce a relatively uniform distribution of the protein showed the presence of several gamma-globulins, siderophilin (the iron-binding beta₂-globulin), lipoproteins, albumin, ceruloplasmin (a blue copper-containing alpha₂-globulin), and a complex of hemoglobin with haptoglobin (an alpha₂-globulin).

Other examples of the use of the chromatographic technique have been the isolation of an antibody activity from serum; analysis of the protein of bovine pancreatic juice, 90 percent of which was recovered as discrete components; separation of egg-white proteins; purification of the thyroid-stimulating hormone; and purification of several viruses and rickettsiae. Successful applications of cellulose ion exchanges have been reported with materials ranging in molecular weight from fibrinopeptides (about 2000) to molecules as large as tobacco mosaic virus (millions).

Drs. Sober and Peterson conclude that the technique is constantly improving in precision and has provided a tool that can be expected to grow in usefulness.

HUMAN GAMMA GLOBULINS FOUND TO COMPRISE TWO MAJOR PROTEIN GROUPS

Dr. John L. Fahey, General
Medicine Branch, has reported
a study in which he used the
Sober-Peterson technique for

the separation and partial characterization of a spectrum of normal gamma globulins from human blood. This makes possible more detailed studies of the nature of these proteins which include the antibodies of normal serum.

In this study, the gamma globulins were isolated from the rest of the normal blood serum proteins. Fractionation was then carried out by the technique of anion-exchange cellulose chromatography. Five fractions were obtained and studied in detail.

Fractions 1-4, comprising about 90 percent of the material, contained proteins of the same molecular size and antigenic properties. These fractions differed, however, in electrical charges and carbohydrate content. They also differed in antibody content, fraction 1 containing the antibodies to mumps virus, Histoplasma capsulatum and certain typhoid bacterial components.

The fifth fraction contained the very large gamma macroglobulins present in small amount in normal serum. These proteins had more carbohydrate and a second antigenic component which helped to distinguish them from the smaller molecules which form the bulk of the normal gamma globulins. The fifth fraction contained most of the Rh antibodies, the characteristic serum factor found in rheumatoid arthritis and other distinctive antibodies.

Dr. Fahey concludes from these observations that the gamma globulins are composed of two groups of proteins, which differ on the basis of size and other properties. At least

one of these groups is comprised of a spectrum of protein molecules having similar size and antigenic properties but differing in antibody content and physicochemical characteristics. Anion-exchange cellulose chromatography is a useful means of subdividing the serum gamma globulins.

CANCER DETECTION

CYTOLOGY IS USEFUL IN DETECTING CANCER OF DIGESTIVE TRACT

Exfoliative cytology has proved to be an effective technique in the detection of certain malignancies,

such as uterine cancer. The results of a 3-year study in which this technique was applied to 1,561 patients with suspected cancer of the gastrointestinal tract have now been described by Dr. Howard F. Raskin and his associates at the University of Chicago School of Medicine.

Cell washings were obtained from four major areas--esophagus, stomach, pancreatic and biliary systems, and colon--and examined microscopically to detect the presence of malignant cells.

Of 151 patients who were examined for cancer of the esophagus, 69 were found to have carcinomas and 66 (95 percent) were established preoperatively by means of the cytologic examination. The stomach was the most frequently examined organ and in 871 cases studied, 125 out of 131 (95 percent) were correctly diagnosed cytologically. Comparison of cytologic and X-ray results showed that cytology was somewhat more accurate for the detection of gastric cancer. However, because of the time required for this test, the investigators suggest that cytology may be held in reserve for special cases.

The pancreatic and biliary systems were the most difficult to study because the duodenum is relatively inaccessible and exfoliation of cells is scanty. Nevertheless, of 356 duodenal drainages performed, 55 proved carcinomas were encountered; malignant cells were recovered in 33 (60 percent) of these patients.

Colonic washings of 183 patients were examined cytologically. Of 38 clinically proved malignancies, the cytologic test was positive in 36 (95 percent).

False positive results were infrequent in esophagus, stomach, and duodenal areas and none were obtained in examinations of the colon.

It was concluded that the exfoliative cytologic technique is an effective aid in the diagnosis of cancer of the digestive tract.

CELLS UNIFORMLY SPREAD
ON CYTOANALYZER SLIDES
BY NEW SPRAY TECHNIQUE

Previous reports by scientists of the Field Investigations and Demonstrations Branch have described the progress in

development and preliminary use of the cytoanalyzer. This automatic scanning instrument was devised to speed the examination of specimens from vaginal-cervical fluids for the presence of malignant cells.

One of the greatest single obstacles in the development of this instrument has been the lack of a satisfactory method for preparing the slides for analysis. Dr. John C. Pruitt has now reported development of a spray technique that produces a single layer of cells on the slide. The technique involves fixation of the aspirated cells in solution and sieving the specimen. It is then placed in an atomizer and sprayed onto a glass slide. The slide is strained and examined. This technique gives excellent distribution of cells on the slide without damaging them.

NEW PROGRAM CREATED
TO DEVELOP METHODS
OF DETECTING CANCER

A comprehensive diagnostic research program has been established within the Field Investigations and Demonstrations

Branch to develop new methods of cancer detection and to improve and extend existing techniques in this important field. The program employs a broad, interdisciplinary approach and is being carried out through direct cooperative activities, contractual arrangements, and grants.

The recognition that cancer is a group of diseases with a variety of clinical manifestations suggests that efforts for improving cancer diagnosis should be aimed toward development of at least several diagnostic procedures rather than a single one. Furthermore, the importance of the cancer-bearing host is pointed up by the fact that although cancer is widespread, only a fraction of the total population is susceptible to the disease. Hence, research on approaches to better cancer diagnosis must include also the study of host factors and host-tumor relationships, including attempts to understand possible host immune factors and other biological or biochemical mechanisms within the host that make it possible for cells to grow in an uncontrolled manner.

The first investigations under the program have been initiated with studies designed to search for measurable differences between the normal and cancerous states. As of December 1, 1959, ten contracts and several direct cooperative projects were in effect.

Biochemical studies are being conducted to determine differences in distribution of enzymes in the blood serum of cancer patients and other persons. Tests are also under way to determine the distribution patterns in blood serum of other biochemical components, such as antibodies, and protein and carbohydrate fractions.

Studies are being carried out to determine the characteristics and significance of malignant cells in the peripheral blood. Investigations are also in progress to learn more about the lining of the uterus in women with cancer of that site. Other studies are concerned with the development of fluorescein dyes with which to identify cancer cells and the application of fluorescent microscopy to the study of malignant gastric cells.

RADIATION RESEARCH

STUDIES PROVIDE BASIC
INFORMATION ON HOW
RADIATION AFFECTS CELLS

Knowledge of the sites and
mode of action of radiation at
the cellular level is particularly
important in cancer research,

which is seeking information on the carcinogenic and carcinocidal effects of radiation.

Scientists of the Radiation Branch have been investigating the effect of radiation on yeast cells in an effort to identify the molecular and anatomical sites sensitive to lethal irradiation. Dr. Mortimer M. Elkind has now reported that dividing yeast cells are slightly less sensitive to ultraviolet (UV) radiation than are nondividing cells. This finding is similar to that reported in the scientific literature some years ago showing that dividing yeast cells are much less sensitive to X-rays (X) than are nondividing cells.

The existence of this similarity in sensitivity to radiation makes it plausible that UV lethal sites overlap X lethal sites in dividing cells. A study was therefore undertaken to examine the existence and degree of overlap of such sites by the use of various combinations of UV, X, and visible light exposures on three yeast cell lines. The rationale for this procedure was based first on evidence implicating the nucleus as the region of primary X damage and second on observations that UV-X coupling effects that are reversed by visible light in all likelihood involve nuclear nucleic acid as the sites common to both irradiations.

Dr. Elkind noted that there appears to be considerable overlap in sites sensitive to X, UV, and photoreactivation (visible-light reversal of UV lethality). In addition, he observed a

new effect; namely, that X lethality is mitigated on the dividing moiety of the population by small UV exposures administered before, during, or after x-radiation. These results support the general conclusion that the nucleus is the location of the UV-X and the X-UV effects observed in this study and that the sites of action consist of the nuclear nucleic acid.

EFFECT OF IRRADIATION
ON METASTASIS OF CANCER
STUDIED IN LABORATORY

A laboratory study of the effect of irradiation on the dissemination of cancer was undertaken by Dr. Peter D. Olch, Surgery Branch,

as a result of observations that some patients show rapid development of distant metastases for the first time shortly following surgical or irradiation treatment of their primary tumor.

The plan of the experiments was to investigate the viability in mice of irradiated tumor cells, as manifested by their growth as artificial pulmonary metastases. (Artificial pulmonary metastases is the term used for lung tumors resulting from the intravenous inoculation of a tumor cell suspension.)

S91 melanoma was inoculated intramuscularly into adult mice; three weeks later the intramuscular tumors were irradiated in vivo with 3000 roentgens (r). At intervals varying from 3 hours to 20 days thereafter, cell suspensions of irradiated tumors were injected into the tail vein of normal mice. As a control, non-irradiated tumors were injected intravenously into mice on the 1st, 11th, and 20th days.

The number of the artificial metastases was smaller than that observed in control animals for three days and the size of such metastases was smaller for eight days. Subsequently the number and size of metastases gradually increased to the level of the controls.

Dr. Olch interpreted these results to mean that this particular tumor is affected in some way by irradiation either immediately or within hours, resulting in a temporary inhibition of the tumor cells' capacity to become established as pulmonary implants. The decrease in number of pulmonary tumors may be an indication that the host is resistant to the irradiated cells, or possibly that the irradiated cells are non-viable.. The suggested inhibition of growth potential is gradually lost as the time between irradiation and implantation of tumors increases.

WHOLE-BODY RADIATION
PRODUCES DIFFERENT
RESPONSES IN ANIMALS

An understanding of the effects of whole-body radiation is of great importance in view of the extensive efforts in progress

in tests of nuclear weapons and investigation of outer space. Results of a study reported by Dr. Howard L. Andrews, Radiation Branch, are, therefore, particularly timely in contributing new knowledge of the response of different species of laboratory animals to high energy, whole-body radiation.

Survival data obtained on mice, rats, guinea pigs, and hamsters given X-ray doses of 5 to 100 kiloroentgens (kr) showed striking differences. In rats and hamsters, there was a gradual decrease in survival time with increase of radiation. There was a gradual trend toward central nervous system hyperexcitability, but even at 100 kr the animals showed little violent convulsive activity. Pentobarbital had no effect on survival time.

Guinea pigs and mice, on the other hand, showed sharp discontinuities in the dose-survival time curves. In guinea pigs, this effect was partially suppressed by administration of pentobarbital before radiation, but the drug had no effect on the survival time of mice at any radiation dose tested.

The results of shielding the head of mice suggested that the radiation received directly by the head had a negligible effect below the transition dose, but was very important above it. Death at high doses appeared to be related to central nervous system injury.

MARROW PLUS METHOTREXATE
EXTENDS SURVIVAL OF MICE
AFTER LETHAL IRRADIATION

Protection of lethally irradiated mice by intravenous inoculation of bone marrow, first observed at the National Cancer Institute,

has been demonstrated by numerous investigators. Isologous marrow, marrow from a member of the same inbred strain, produces a lasting recovery in irradiated mice, although their life span is shortened. Homologous marrow, marrow from a different strain of the same species, usually produces only temporary protection; the mice survive acute radiation damage but succumb during a secondary phase of the irradiation syndrome. This secondary or homograft reaction is generally regarded as the result of an immune response of the host to the tissue of the foreign strain.

Miss Delta E. Uphoff, of the Laboratory of Biology, has reported that the homograft reaction is suppressed or its lethal effect nullified by administration of the drug, methotrexate. Hybrid mice, 3 to 4 months old, received 800

roentgens of total-body X-irradiation, a dose lethal to 98 percent of mice of this strain, followed by homologous bone marrow. Treatment with methotrexate was started 14 days later. When the animals had survived acute irradiation damage, the body weights had returned to pre-irradiation levels, and the homograft reaction had not set in. Survival was increased to 70-90 days after irradiation, as compared with a maximum survival of 38 days in animals that did not receive methotrexate.

Different treatment schedules produced effects ranging from a typical homograft reaction to no detectable reaction.

Further work on the nature of the action of methotrexate in suppressing the homograft reaction in irradiated mice is in progress.

100 SCIENTISTS ATTEND RADIATION CONFERENCE

The National Cancer Institute was host in September to a research conference on the clinical aspects of radiation injury and protection from radiation. Over 100 scientists from some 50 institutions in the United States and abroad participated in the meeting, which was one of a series begun in 1956 by the Biology Division of the Oak Ridge National Laboratory to stimulate discussions and exchange of ideas among investigators doing basic research on radiation protection and the clinical scientists who apply their findings.

Dr. Charles G. Zubrod, Clinical Director, National Cancer Institute, presided at the opening session, which was devoted to "Chemical protection against radiation and alkylating agents." The afternoon session, a discussion of "Transplantation of bone marrow and other organs," was presided over by Dr. E. Donnall Thomas, The Mary Imogene Bassett Hospital, Cooperstown, N. Y. The concluding discussion on "Germ-free life for the totally radiated patient," was moderated by Dr. J. W. Ferrebee, also of The Mary Imogene Bassett Hospital.

One of the most significant findings reported to the group was that the lethal radiation dose for man is much higher than formerly believed. This knowledge could be of considerable benefit in the management of the irradiated patient. A significant development in bone marrow transplantation was the technique of removing large quantities of bone marrow from the patient before radiation, quick-freezing the marrow, and giving it back to the patient after irradiation as needed. Chemical

protection against the effects of radiation has been observed in laboratory animals; similar studies in man indicate a need for development of drugs that are less toxic.

LEUKEMIA STUDIES

REGIMENS OF 6-MP PLUS METHOTREXATE COMPARED BY COOPERATIVE GROUP

A comprehensive clinical investigation comparing two regimens of combination chemotherapy of acute leukemia

was conducted by scientists of the General Medicine Branch and other investigators of the National Cancer Institute, Roswell Park Memorial Institute, and Children's Hospital of Buffalo. These scientists constituted one of the cooperative clinical groups organized by the Cancer Chemotherapy National Service Center.

A detailed protocol was designed specifying the procedure under which the therapeutic programs were applied and evaluated. Sixty-five patients received the same total doses of 6-mercaptopurine (6-MP) and methotrexate concurrently. Thirty-three patients on "continuous" therapy received daily doses of both drugs; 32 patients on "intermittent" therapy received 6-MP daily and methotrexate every third day. When remissions occurred, patients were placed on maintenance therapy at one-half the dose that produced toxicity.

Dr. Emil Frei, National Cancer Institute's General Medicine Branch, has reported that there was no appreciable difference in results between the two regimens with respect to frequency of remission (number of patients in a group who satisfied the criteria for partial or complete remission), extent of remission (either partial or complete), amount of drug administered, days to toxicity, and qualitative manifestations of toxicity. However, among patients who attained remission status, duration of remission and survival were longer for the "continuous" than for the "intermittent" group.

Among the children, the median survival from symptomatic onset of acute leukemia was 12 months. The median survival for adults was 7 months.

All remissions in children occurred in acute lymphocytic leukemia and all remissions in adults occurred in acute myelocytic leukemia. The frequency of remission, either partial or complete, was higher in children (36 percent) than in adults (19 percent).

Toxic manifestations were qualitatively and proportionately the same in patients whose disease remitted and in those whose disease did not remit. No confirmation was obtained, therefore, for the suggestion that administration of drugs to maximum tolerated doses might produce higher remission rates.

Until other, more effective antileukemic treatment is discovered, detailed studies such as the present one using drugs currently available are important to determine how these agents can be used to produce optimum results, Dr. Frei noted.

HIGH LEUKEMIC CELL COUNT IN BLOOD ASSOCIATED WITH INTRACRANIAL HEMORRHAGE

Intracranial bleeding is a common cause of death in acute leukemia. This often occurs as part of a general

tendency for leukemic patients to bleed spontaneously from many different blood vessels and is accompanied by marked diminution of the concentration of blood platelets in the circulating blood. Scientists of the General Medicine Branch and Pathologic Anatomy Branch have described another factor associated with massive intracranial hemorrhage, namely rapidly increasing numbers of leukemic cells in the peripheral blood.

The report by Dr. Richard D. Fritz and his associates details studies on 100 patients with acute leukemia, of whom 81 had died. Fatal intracranial hemorrhage was the terminal event in 69 percent of the 13 patients whose white blood count exceeded 300,000 and occurred within a few days of onset of this marked leukocytosis. These patients had significantly higher platelet levels, less marked spontaneous bleeding, and a shortened overall survival than patients with acute leukemia who died of intracranial hemorrhage with leukocyte counts less than 300,000. In addition, the hemorrhage which predominantly involved the white matter of the brain was related to a distinctive lesion consisting of nodules of leukemic cells which apparently had injured the intracerebral vessels.

Only 13 percent of the remaining 68 patients whose blood counts were less than 300,000 died of intracranial hemorrhage. The hemorrhage in each instance was subarachnoid or subdural, as seen in non-leukemic patients.

FRESH BLOOD BETTER THAN PRESERVED IN TREATING HEMORRHAGE OF LEUKEMIA

Scientists of the General Medicine Branch and the Cancer Chemotherapy National Service Center have collaborated with

scientists of the Division of Biologics Standards in a study of the efficacy of transfusions of fresh blood compared with preserved whole blood in the treatment of hemorrhage in a small group of leukemia patients.

Data were obtained on the results of 16 transfusions administered to 6 patients. Eight of the transfusions were of fresh blood and eight were of preserved blood. Four of the patients showed a clinical effect on hemorrhage, either cessation or diminution of bleeding. All of these followed transfusions of fresh blood.

Dr. Emil Freireich, General Medicine Branch, concludes from these results that fresh blood is more effective than preserved whole blood in alleviating gross hemorrhage in patients with acute leukemia.

PATIENT SUSCEPTIBILITY
TO BACTERIAL INFECTION
RELATED TO USE OF DRUGS

One of the consequences of the advent of potent antibiotics and other new drugs has been the increased importance of

Pseudomonas aeruginosa as an infectious agent in man. Scientists of the General Medicine Branch and Pathologic Anatomy Branch, collaborating with investigators of the National Institute of Allergy and Infectious Diseases, have studied some of the clinical, therapeutic, and morphologic aspects of pseudomonas septicemia in a number of patients at the Clinical Center,

Dr. Claude E. Forkner, Jr., General Medicine Branch, reported that twenty-two out of a group of 23 patients observed between May 1954 and January 1957 died of pseudomonas septicemia. The first positive blood cultures had been obtained four days previous to the deaths. All but two of the patients had malignant disease and 13 had acute leukemia.

Fifty-two percent of the patients developed jaundice, 65 percent had neurological disorders, and 39 percent had cutaneous lesions. An affinity of the Pseudomonas aeruginosa microorganism for the walls of the small blood vessels was observed.

In other patients with various bacterial septicemias, Pseudomonas aeruginosa was second to Staphylococcus aureus as a cause of septicemia and the most frequent bacterial cause of death in the leukemia service of the Clinical Center. Furthermore, pseudomonas septicemia frequently occurred as a superinfection among patients receiving broad-spectrum antimicrobial therapy.

Among patients who had received antimetabolites, deaths occurred more frequently from pseudomonas septicemia than from other bacterial septicemias. Dr. Forkner suggests, therefore, that the administration of antimetabolites in treatment of leukemia in some way increases the susceptibility of patients to pseudomonas septicemia.

INVESTIGATIONS CONTINUE ON SERUM B-12, LEUKEMIA

Scientists of the General Medicine Branch have previously described studies in a large series of patients confirming the fact that serum vitamin B-12 concentrations were higher in patients with chronic myelocytic leukemia than in patients with other types of cancer or well persons. (Highlights of Progress in Research on Cancer, 1958, p. 15). Dr. I. B. Weinstein has now reported the results of studies performed to shed more light on this phenomenon.

In these, radioactively labeled B-12 was added to samples of serum taken from chronic myelocytic leukemia (CML) patients and normal subjects. A series of analytical procedures showed that the B-12 was bound to the fraction of serum protein called seromucoid. The seromucoid in the CML patients bound substantially more of the vitamin than did that of the controls.

Studies still in progress indicate that the serum seromucoid is chemically related to "intrinsic factor," a constituent of gastric juice that facilitates the passage of B-12 from the stomach into the blood stream.

At present, the significance of increased amounts of B-12 binding protein in the serum of the CML patients is not known. If future research shows that the growth of leukemic cells is associated with increased utilization of B-12, the importance of the increased binding capacity of leukemic serum in the perpetuation of the leukemic process will be evident.

WHOLE-BODY IRRADIATION, BONE MARROW INFUSION GIVEN LEUKEMIA PATIENTS

Infusion of normal bone marrow will induce recovery in several animal species after exposure to irradiation that would otherwise prove to be lethal. In man, there is little quantitative information regarding the effects of whole-body irradiation and only preliminary information is available concerning marrow transplantations after irradiation.

Investigators at the Mary Imogene Bassett Hospital, Coopers-town; New York, and the Children's Cancer Research Foundation, Boston, Massachusetts, have studied the effects of bone marrow transplantation following whole-body radiation treatment of 6 leukemia patients.

Whole-body irradiation was given in divided doses and the estimated average tissue dose ranged from 250 to 600 r.

Bone marrow was obtained from either close relatives or individuals with similar blood types and was given intravenously soon after radiation treatment was completed. One patient also received fetal hematopoietic tissue.

Dr. E. Donnell Thomas has reported that most of the patients felt better in the immediate post-radiation period. However, only two were considered to have obtained significant clinical benefit. All but one of the patients died from bacterial infections. The one patient who survived at the time the report was written had been maintained in sterile isolation. In this case, a bacterial infection occurred and was successfully controlled. A follow up study disclosed that approximately 7 months after total body irradiation and bone marrow transplantation this patient remained clinically well.

Dr. Thomas suggests that the immediate biologic problem after whole-body irradiation is the management of infection. Bone marrow transplantation does not appear to restore the antibacterial defenses. He concludes that much research needs to be done on the development of radiation facilities to produce homogenous ionization effects in the human body and on the application of bone marrow transplantation to assess its immunological and other effects.

EMBRYONIC LIVER TISSUE
PROTECTS IRRADIATED MICE
FROM LEUKEMIA DEVELOPMENT

Scientists of the Laboratory of Biology have been studying the development of leukemias in laboratory animals following exposure to ionizing radiations.

Previous studies reported by Dr. H. S. Kaplan, Stanford University, showed that genetically compatible bone marrow, protected first-generation hybrid mice against the development of radiation-induced lymphomas. In the study now reported, the protective effects of bone marrow and fetal blood-forming tissue were compared.

Fetal blood-forming tissue was selected because previous observations at the National Cancer Institute had shown that such tissue afforded mice the same excellent protection from death due to radiation as did isologous marrow, (marrow from the same strain). (Highlights of Progress in Research on Cancer, 1958, p. 24).

Miss Delta E. Uphoff has reported that a group of first-generation hybrid mice, 1 month old, was exposed to a total of 672 roentgens of X-radiation given in 4 equal doses at 7-day intervals. Shortly after the final irradiation, test

mice received intravenous inoculations of embryonic liver tissue or bone marrow. At 13 months of age, the incidence of lymphomas was as follows: among the controls, which received no protective treatment, 64 percent; among the group receiving bone marrow from a parent strain, 22 percent; and among the group receiving embryonic liver tissue, 34 percent.

Miss Uphoff concludes that lymphomas can be prevented to some extent in irradiated mice by injection of embryonic liver tissue. However, the protective effect is probably not equal to that produced by inoculation of marrow from the same or a parent strain.

STUDY TUMOR VARIANTS OF MOUSE LEUKEMIA

Scientists of the Laboratory of Chemical Pharmacology have previously reported that mice

with systemic leukemia L1210 survive an average of 90 days when treated with halogenated derivatives of methotrexate (amethopterin); this is nine times the average survival time of untreated mice. Some mice given extensive therapy developed recurrences at various sites. Transplantation of tissue obtained from such mice produced tumor variants of leukemia L1210.

Mr. Stewart R. Humphreys reported that the tumors were histologically similar to the original L1210 but showed a wide range of differences in rates of growth, degree of invasiveness, and response to drugs, including high sensitivity as well as almost complete resistance. There appeared to be little correlation between the rate of tumor growth and degree of response to drugs. The variants appeared to be relatively stable in serial passage, without further treatment. One of the slow-growing variants that attained large sizes and showed long survival times retained these characteristics after 15 generations.

Mr. Humphreys concludes that the study of tumor variants such as these will facilitate studies of the phenomenon of drug resistance and help produce a better understanding of host-tumor-drug relationships, which govern drug effectiveness in cancer chemotherapy.

CHEMOTHERAPY RESEARCH

AET PREVENTS RADIATION TOXICITY BY ACCUMULATING IN SENSITIVE NORMAL TISSUE

Earlier reports have indicated that a chemical, AET, protects mice against toxic effects of radiation and nitrogen mustard.

Treatment with the chemical does not suppress the anticancer

effects of either X-rays or nitrogen mustard. (Highlights of Progress in Research on Cancer, 1958, p. 38).

The compound, S,2-aminoethylisothiuronium bromide hydrobromide (AET), was synthesized in 1955 by Drs. David G. Doherty and W. T. Burnett, Jr., Biology Division of Oak Ridge National Laboratories, Oak Ridge, Tennessee. An investigation in 1957 of the toxicity and therapeutic effectiveness of AET in man by Dr. Paul T. Condit, General Medicine Branch, and coworkers, showed that the chemical was toxic and that at the doses employed, no response of skin or hair to radiation was observed.

A reason for the differences in reactions of the normal and neoplastic tissues of mice has now been reported by Dr. Roger W. O'Gara, Laboratory of Pathology, who collaborated in the study with Joanne W. Hollcroft, Radiation Branch, and Dr. Margaret G. Kelly, General Medicine Branch. The findings may explain why AET permits treatment of tumor-bearing mice with ordinarily lethal doses of radiation.

AET labeled with radioactive sulfur was found to concentrate especially in liver, intestine, spleen, and bone marrow when injected into normal or tumor-bearing mice. The tumors and their metastases took up little of the drug. An autoradiograph of a liver containing metastatic cancer revealed little or no radioactivity in the cancerous area, but considerable isotope in the adjacent, relatively normal hepatic tissue.

The bone marrow and intestinal mucosa are particularly sensitive to damage by ionizing radiations. AET in these tissues probably counteracts the local effects of X-rays which contribute to lethality. Failure of cancers to accumulate any substantial amount of the drug permits X-rays to produce the damage which is therapeutically desirable.

STUDY ON DYE METABOLISM
MAY HELP IN UNDERSTANDING
MECHANISM OF DRUG ACTION

Pharmacological studies of
antitumor drugs and other
substances may shed light on
the mechanism of action of

chemotherapeutic agents. One recent study has produced information on the metabolism of phenol red, a dye used in staining cells for microscopic examination.

Dr. Ti Li Loo, General Medicine Branch, and Dr. J. Wendell Burger, of Trinity College, Hartford, Conn., have reported that the uterus of the pregnant spiny dogfish can convert phenol red into a purplish-blue dye, bromophenol blue. This is the first example of biological bromination that has been observed in a vertebrate.

In the experiments phenol red dissolved in sea water was introduced into the uteri of dogfish in the second summer of pregnancy. In the first summer, the eggs in each uterus are enclosed in a common capsule, with the uterine wall in close apposition to the capsule. The capsule is broken and in the second summer the embryos--small fish with yolk sacs, called pups--lie free in the uterine cavity. After 24 to 48 hours, all or most of the phenol red was converted to the purplish-blue dye, later identified as bromophenol blue, in each of the eight fish tested.

Further studies are in progress to determine the mechanism of the bromination, which the authors suggest may be catalyzed by an enzyme system.

PENETRATION OF DRUGS THROUGH BLOOD-BRAIN BARRIER DESCRIBED

Knowledge of the factors which influence the selective penetration of drugs from blood to cerebrospinal fluid (CSF) is important for the

rational use of antitumor agents in the treatment of patients with tumors located in and around the brain.

Scientists of the General Medicine Branch are investigating this aspect of the pharmacology of antitumor drugs. In one study, Dr. David P. Rall has described a general method for the quantitative study of blood-CSF transfer kinetics in dogs and reported on the distribution of certain weak organic electrolytes between blood and CSF.

The drugs passed from blood to CSF to reach a steady state ratio characteristic of the drug according to its pK_a (ionization constant) and of the normal pH gradient between blood and CSF. A drug that was almost completely ionized at body pH (7.4) was excluded from CSF. Drugs that were completely unionized at body pH were distributed equally between blood and CSF. Drugs that were partially unionized at body pH entered CSF to an extent dependent upon the degree of ionization of the compound and upon the acidity or alkalinity of the compound. Normally, blood pH is more alkaline than CSF pH and this pH gradient affects drug distribution. Weak acids were partially excluded from CSF, while weak bases would tend to be concentrated in CSF. The hypothesis that pH gradients between blood and CSF affect the distribution of these drugs was supported by the observation that under conditions of altered pH gradients between blood and CSF, the steady state distribution ratio of partially ionized drugs was significantly altered. The rate of drug entry into CSF seemed to be dependent upon lipid solubility determined at pH 7.4. The blood-CSF barrier thus

appears to have properties very similar to other biological membranes and is much less of a specific barrier than has been postulated.

In a second study, Dr. Charles G. Zubrod has reported on the distribution of drugs between blood and CSF and brain in the five vertebrate classes. Representative species selected for examination showed three possible anatomical variants of the meninges. Fish have a single meningeal layer; considerable ventricular fluid (VF) which is comparable to mammalian CSF; and an abundance of extradural fluid (EDF). Reptiles and amphibia have two layers with small amounts of fluid between them. Although birds and mammals have three layers, mammals have a large amount of subarachnoid fluid, while birds have but a small quantity.

In general, all the species studied behaved uniformly in transferring sulfanilamide and endogenous ascorbic acid into the brain and in excluding sulfanilic acid from the brain. In the frog and dog, the results were similar with respect to transfer of drugs into CSF. In the fish, sulfanilic acid was excluded from VF, thus suggesting uniformity in the blood-CSF barrier throughout the vertebrates. The extent to which drugs were transported into fish EDF suggests that no barriers exist between this fluid and blood. These observations suggest that fluid outside the meninges does not have the characteristics of CSF.

MEASUREMENT OF TUMORS SATISFACTORY IN JUDGING EFFECT OF ANTITUMOR DRUGS

In order to test the effects of chemotherapeutic agents in patients with cancer, it is necessary to have methods

for following changes in tumor growth. Indirect methods of measuring changes in excretion of metabolic products and in serum levels of certain enzymes may in some instances reflect changes in tumor activity, but such methods are not generally applicable at the present time. Dr. Clyde O. Brindley, General Medicine Branch, has described his observations of the direct measurement of tumors.

Eighty-six advanced cancer patients with measurable tumors were selected for the study. Skin and subcutaneous tumors were measured directly with calipers at weekly intervals while lung and bone lesions were recorded by roentgenograms at two-week and three- to four-week intervals, respectively. The lesions measured were discrete in outline, accessible, and included as many cell types as possible.

Dr. Brindley concludes that direct measurement of tumor masses or measurement on standard roentgenogram, together with observations of changes in the number of metastases, constitutes a satisfactory method of assaying tumor growth.

ISOLATION-PERFUSION CHEMOTHERAPY GIVES PROMISING RESULTS

A group of investigators at Tulane University, Charity Hospital, and the U. S. Public Health Service Hospital,

New Orleans, La. have reported on the use of an isolation-perfusion technique for administering anticancer agents to patients with various types of malignancy. Essentially the technique involves isolating the tumor-bearing area (arm, leg, pelvis, etc.) from the normal circulation stream and introducing into the area relatively high concentrations of anticancer drugs. In addition, a method of total body perfusion has been developed and utilized for treating disseminated cancers.

Research on this technique stems from two obstacles in clinical cancer chemotherapy: 1) no truly specific carcinocidal agents are presently available; and 2) existing drugs are toxic to rapidly growing normal tissue when administered in therapeutically effective doses.

In the study, 73 patients were treated by isolation-perfusion from June 1957 through November 1958. The types of cancer included tumors of the breast and genitourinary system, malignant melanoma, and osteogenic sarcoma. Five anticancer drugs were employed, either singly, in combination, or as adjuvants to surgery. These were: nitrogen mustard, phenylalanine mustard (PAM), actinomycin-D, triethylenethiophosphoramide (TSPA), and 5-fluorouracil (5-FU).

At the time the report was written (December 1958), 22 of the patients were alive and clinically free of disease, 8 were alive with disease that had undergone regression and appeared to be static, 13 were dead, and 30 had recurrent disease. Included in this last category were patients who failed to respond to treatment, whose lesions temporarily responded and then recurred, or who had been treated too recently for response to be evident.

The most frequent and troublesome complication resulting from isolation-perfusion therapy in this study was the development of edema in the extremities. Less serious complications included reversible depression of hemopoiesis and gastrointestinal upset.

Dr. Oscar Creech, Jr., concludes that some human tumors can be controlled through isolation-perfusion chemotherapy

employing high concentrations of anticancer agents. It is apparent, however, that great variation exists in the response of tumors to anticancer drugs and that the maximum safe amount of drug that can be administered by this technique has not been determined. He adds that it is difficult to compare the value of isolation-perfusion with the conventional methods of administering anticancer drugs, but suggests that if the right combination of factors could be brought to bear on a tumor and its immediate environment, total destruction of the tumor could be possible without subjecting the entire body to the toxic effects of the agent.

NEW RIBOFLAVIN COMPOUND
INEFFECTIVE AS TREATMENT
FOR DISSEMINATED CANCER

Previous studies have shown that restriction of the vitamin, riboflavin, from the diet of mice and rats has resulted in

regression of tumors. Attempts to induce riboflavin deficiency in man by means of a restricted diet have been generally unsuccessful. Several riboflavin antagonists have been tested including one that was given orally (Highlights of Progress in Research on Cancer, 1958, p. 41), and failed to induce riboflavin deficiency. Parenteral injection of that compound was hampered by its insolubility in aqueous solution.

A group of investigators of the Institute's General Medicine Branch and the Upjohn Company Research Laboratory, Kalamazoo, Michigan, collaborated in the synthesis and testing of a water-soluble riboflavin antagonist, and Dr. Montague Lane of the National Cancer Institute has now reported the results of their studies.

The compound, sodium-6, 7-dimethyl-9-(2'-hemisuccinoxyethyl)-isoalloxazine (U-6538), was prepared in 1958 by Dr. Harold G. Petering at Upjohn. When injected intraperitoneally, the drug was capable of inhibiting the growth of rat lymphosarcoma and was well tolerated by rats and dogs even after prolonged administration.

Four patients with disseminated cancers were given intravenous or intramuscular injections of the drug. All the patients excreted in the urine a large proportion of the unchanged drug and a smaller proportion of a relatively insoluble metabolite, U-2113, which crystallized in the urinary tract and produced acute renal toxicity. At maximally tolerated doses, no definite riboflavin deficiency was induced by U-6538 and no antitumor effect was noted in any of the patients.

CYTOXAN SUBSTANTIALLY INCREASES SURVIVAL TIME OF MICE WITH LEUKEMIA

Laboratory tests of a new alkylating agent, cyclophosphamide, have shown that it is markedly effective in increasing the

survival time of mice with advanced L1210 leukemia. Dr. Montague Lane, General Medicine Branch, has reported that cyclophosphamide produced up to 200 percent increase in median survival beyond that of untreated, control animals.

Cyclophosphamide [N,N-bis-(beta-chloroethyl)-N', O-propylene phosphoric acid ester diamide] is a member of the class of compounds known as "nitrogen mustards". It was developed in the laboratories of Asta-Werke, A. G., West Germany, in 1957, and its synthesis reported in the scientific literature by Arnold and Borseaux in 1958. The effectiveness of the compound against animal tumors was reported in 1958 in a series of papers by Arnold, Borseaux, and Brock. Initial clinical investigations were conducted at the Universities of Marburg, Duisberg, and Kiel, and preliminary reports on the usefulness of the drug as an anticancer agent published in the German literature in 1958 by Gross and Lambers, and others. The compound is known as B-518 in Europe, and is sold there by Asta-Werke under the name of Endoxan. In this country, it is marketed as Cytosan by Mead Johnson and Company, Evansville, Indiana. Within the past few weeks, Mead Johnson has distributed technical information about cyclophosphamide to physicians throughout the country. The compound is being studied under the program of the Cancer Chemotherapy National Service Center and in the intramural research program of the National Cancer Institute.

In the present study, the drug was most effective when given once a week beginning 5 or 7 days after the tumor was implanted in the test animal. Several mice were alive 35 days after tumor implantation; untreated mice had a median survival of 9 to 10 days.

The drug was equally effective when administered by oral, subcutaneous, or intraperitoneal route. The optimum dose for oral treatment was higher than for either parenteral route. Toxicity was in general similar to that produced by nitrogen mustard.

Dr. Lane concluded that cyclophosphamide was much more effective than the antifolic agent methotrexate, or the alkylating agent, nitrogen mustard, in prolonging the survival of leukemic mice when the optimum dosage schedule for each agent was used. A strain of leukemia resistant to 6-mercaptopurine was as susceptible to cyclophosphamide as the original strain.

HORMONE IN ITS PROPIONATE
FORM USED TO TREAT HUMAN,
METASTATIC BREAST CANCER

changes in metastatic lesions. Various compounds have been tested in an effort to prolong the period of regression, but so far none has exceeded testosterone propionate (TP) in anticancer effect.

In treating certain patients with breast, androgenic hormones have been found capable of inducing temporary regressive

In an effort to enhance the anticancer ability of TP, structural alterations of the drug have been made. One steroid resulting from such changes is 2 alpha-methyl-androstan-17 beta-ol, 3-one (2-methyl dihydrotestosterone), which was synthesized in 1956 by Dr. H. J. Ringold and his associates at the Research Laboratories of the Syntex Corporation, S. A., Mexico City. Dr. Charles B. Huggins, Ben-May Laboratory, Chicago, found that the compound inhibited development of a transplantable rat mammary tumor.

One of the clinical study groups organized by the Cancer Chemotherapy National Service Center has investigated the use of the compound in the form of its propionate, 2-alpha-methyl dihydrotestosterone propionate (2M-DHTP), for the treatment of metastatic carcinoma of the breast in a small group of patients. Dr. Charles M. Blackburn, Mayo Clinic, Rochester, Minn., has reported that, of 48 postmenopausal women who had not previously received steroid therapy, 27 were treated with 2M-DHTP and 21 were used as controls and treated with TP, administered intramuscularly.

Temporary regression of metastatic lesions occurred in 12 of the patients given 2M-DHTP, but only in 3 of the patients treated with TP. Subjective observations of the effects of 2M-DHTP suggest that it may be significantly less androgenic than TP.

The results suggest that the androgenic and the anticancer effects of steroidal hormones may not necessarily be inseparably related. Definitive evaluation of the usefulness of 2M-DHTP in the palliative treatment of advanced mammary cancer awaits further clinical experience.

METHOTREXATE DERIVATIVES
BETTER THAN PARENT DRUG
AGAINST MOUSE LEUKEMIA

A systematic program of investigation has been in progress in the Laboratory of Chemical Pharmacology

on the influence of chemotherapeutic agents against transplantable systemic leukemia (L1210) in mice (Highlights of Progress in Research on Cancer, 1958, p. 34). An assay procedure has been developed for this purpose in which treatment is withheld

until the disease is widely disseminated and death is imminent. In the first comparison of a number of antileukemic agents by this procedure, the folic acid antagonist, amethopterin (methotrexate), produced the greatest increase in survival time of mice with advanced leukemia.

Now Dr. Abraham Goldin and his associates have reported on the effectiveness of halogenated derivatives of methotrexate in prolonging the life of leukemic mice. A series of these compounds was synthesized by Drs. Robert B. Angier and William V. Curran in 1958 at the Organic Chemical Research Section of the American Cyanamid Company, Pearl River, New York.

3'-Bromo-5'-chloroamethopterin and 3',5'-dichloroamethopterin, produced median survival times of 90 days and more from day of inoculation with leukemic cells. In one experiment, on the 100th day more than 40 survived in each group of 130 mice treated with one or the other compound. The survival time was 3 to 4 times greater than that observed with methotrexate, which produced a median survival time of 20 to 30 days.

Some animals were still alive 6 months after treatment with the compounds and were presumably "cured." Reinoculation of leukemic cells in these animals failed to produce leukemia, suggesting that they may have become immune.

The results provided an experimental demonstration of the feasibility of long-term control of systemic leukemia by drugs. The dichloro compound is undergoing clinical trial in the National Cancer Institute intramural research program and under the program of the Cancer Chemotherapy National Service Center.

FU DERIVATIVES FUDR, FCDR
GIVE PROMISING RESULTS
IN LABORATORY STUDIES

Clinical trials of the
drug 5-fluorouracil (FU)
have produced sufficiently
promising results to warrant

continued investigation of this agent. Several studies have shown that a rather broad spectrum of solid tumors responds to the drug when it is given until toxicity in the form of stomatitis or moderate diarrhea appears. Objective remissions were observed in patients with cancer of the breast, colon and rectum, cervix, ovary, and liver. Conversely, malignant lesions of the lung, stomach, and pancreas, and malignant melanoma, were unaffected. Since regressions have usually been accomplished only with the production of considerable toxic manifestations, investigators have prepared

several derivatives in the hope of producing the same or better therapeutic activity but less toxicity.

Dr. Joseph H. Burchenal, Sloan-Kettering Institute for Cancer Research, and his associates have now reported that FU and five related compounds--5-fluoroorotic acid (FO), 5-fluorouridine (FUR), 5-fluorodeoxyuridine (FUDR), 5-fluorocytidine (FCR), and 5-fluorodeoxycytidine (FCDR)--were studied against a spectrum of transplanted mouse leukemias.

All the compounds were active against one or more of these leukemias. FU and FUDR were active against methotrexate- and mercaptopurine-resistant lines of leukemia L1210. This finding suggests that these compounds might be of value in patients with leukemias that have become resistant to the conventional agents, methotrexate and mercaptopurine. Dose-response curves on leukemia B82 (an acute lymphatic leukemia) showed that FUDR and FCDR had a relatively high chemotherapeutic index against this type of leukemia. The authors suggested on the basis of these results that these compounds merit clinical trial.

5-Fluorouracil and derivatives are members of a relatively new class of antitumor agents, the fluorinated pyrimidines. Several of these compounds were originally synthesized jointly by Dr. Charles Heidelberger and his colleagues at the University of Wisconsin Medical School, Madison, and by Dr. R. C. Duschinsky and his coworkers at Hoffman-La Roche, Inc., Nutley, New Jersey. Dr. Heidelberger also received support for his investigations from Hoffman-La Roche, Inc. Cytidine derivatives were synthesized by Dr. J. J. Fox and coworkers, Division of Nucleoprotein Biochemistry, Sloan-Kettering Institute for Cancer Research. The work of this Division is supported in part by a National Cancer Institute grant.

At the present time, FU and FUDR (for which Hoffman-La Roche, Inc. is the sole source) are being evaluated in clinical trials under the program of the Cancer Chemotherapy National Service Center and in the intramural research program of the National Cancer Institute.

CHEMOTHERAPY RESEARCH
PROGRAM CONTINUES
FULL-SCALE OPERATION

The research program of the Cancer Chemotherapy National Service Center continued in full-scale operation. Comprehensive

reviews and analyses have been undertaken to insure that all the component parts of the program are operating at the most effective level and to chart the future course, making adjustments necessary to maintain a well-rounded program.

Materials are entering the primary screening process at the rate of 50,000 a year, and increased emphasis is being given to the screening of plant products. An alternate mouse tumor was added during the year to the original three used to assess possible anticancer activity of the test materials and to provide additional information on the few that appear to have potential clinical value. Mice bearing experimental tumors are being used at the rate of 2 million a year. In addition, several other rodent tumors are under consideration as possible additions to the primary screen.

By the end of the year, 109 drugs were being evaluated clinically in studies involving 7,700 cancer patients in hospitals throughout the United States.

Among the experimental anticancer drugs placed in clinical trial within the year, two appeared to be particularly promising on the basis of preliminary results. One of these, 2-alpha-methyl dihydrotestosterone propionate, supplied by Syntex S.A., of Mexico City, produced markedly better results against breast cancer than did its parent compound, testosterone propionate. These results suggest that anticancer activity of drugs related to the male sex hormone may not be inseparably tied to their masculinizing properties. This possibility was further supported by clinical results obtained with another new compound, delta 1 testololactone, which also is chemically similar to the male hormone. This drug, which was prepared by the Squibb Institute for Medical Research, New Brunswick, N. J., also proved of value in the treatment of advanced breast cancer but showed no trace of undesirable masculinizing activity.

As of December, 1959, 106 chemotherapy research contracts valued at more than \$16 million were in effect. Under these agreements, industrial firms, colleges and universities, and other independent research institutions were carrying out essential aspects of the program: producing and testing new drugs, conducting comprehensive research studies, and manufacturing promising agents in sufficient quantities for clinical trial.

The Chemotherapy Service Center sponsored a meeting on experimental clinical cancer chemotherapy, which was held in Washington in November. More than 630 clinicians and other scientists participating in the national chemotherapy program attended the two-day conference. In the main, the speakers agreed that there is need for better correlation between the results of drug testing in animals and clinical trials. They expressed the belief that additional statistical evaluation of screening procedures can probably overcome this

difficulty. Also stressed was the need for further developmental research aimed at producing drugs more effective and less toxic than those now available. Several conference participants called attention to the indirect accomplishments of the program, aside from the development and testing of drugs. Specifically mentioned were the training of large numbers of clinicians to carry out statistically designed clinical studies, the development of close working relationships between scientists of many scientific disciplines, and the accumulation of basic data concerning the natural course of cancer and the effectiveness of current methods of treatment.

At another scientific meeting sponsored by the Chemotherapy Service Center, the broad subject of the relationship of hormones to cancer was reviewed comprehensively by scientists active in the program and other interested investigators. The meeting was held at Vergennes, Vermont, in September. Papers presented included discussions of the role of steroid hormones in the development and growth of malignant disease, the chemistry of these compounds, and recent advances in the hormone treatment of cancer.

OTHER THERAPY

INJECTIONS OF ANTIBIOTIC ENHANCE RESPONSE TO X-RAY THERAPY OF HUMAN CANCER

An enhanced response to radiation was observed in cancer patients who were given X-ray therapy combined

with intravenous administration of actinomycin D. Actinomycin D is an antibiotic isolated by Dr. Selman A. Waksman at Rutgers University, New Brunswick, New Jersey, in 1954. It was used in conjunction with X-rays in this clinical investigation because of earlier findings that the radiation responses in normal tissues were enhanced by a combination of actinomycin D and X-ray. To produce a similar effect in normal structures, doses required in the presence of actinomycin D were smaller than those required in its absence. Actinomycin D alone reactivated latent radiation effects in tissues which were previously irradiated but had returned to normal in appearance.

In the present study, reported by Dr. Guilio J. D'Angio, of the Children's Medical Center and the Harvard School of Medicine, Boston, a group of 126 patients was given 220 courses of combined antibiotic and X-ray therapy. The most favorable response occurred in patients with Wilms' tumor, Ewing's tumor, hemangioendotheliosarcoma, and rhabdomyosarcoma. Definite improvement was observed after 75 percent of the prescribed courses of combined therapy had been administered. Simultaneous

chemotherapy and radiotherapy in low dosage appeared to be as effective as higher doses of either component and there was some evidence of a longer beneficial effect with the combined therapy. No cross resistance between the two forms of therapy was noted even though there appeared to be some similarity in their modes of action.

ILEUM FOUND SATISFACTORY AS URINARY BLADDER IN PELVIC CANCER PATIENTS

Surgical management of patients whose normal urinary flow has been disrupted by disease or operative procedure is a matter

of considerable clinical importance. The successful use of the ileal bladder as a means of urinary diversion in laboratory animals has encouraged scientists of the Surgery Branch to apply this technique clinically. Dr. James R. Jude has reported the results obtained in a group of 22 pelvic cancer patients.

The ileal bladder was constructed by attaching a 20-25 cm. segment of the terminal ileum to the ureter, making an incision in the outer abdominal wall, and allowing the ileal bladder to drain into removable plastic containers. Serial examinations of the blood and urine were performed frequently for evidence of change in physiological processes over periods of six months to two years.

The study revealed that physiological processes were not affected by this method of urinary diversion. Kidney function was maintained or improved, blood electrolytes were not abnormally altered, and the upper urinary tract was not damaged by ascending infection. From these findings Dr. Jude concludes that the ileal bladder is valuable method of temporary or permanent urinary diversion.

LOW VOLTAGE ELECTRIC CURRENT INHIBITS GROWTH OF SARCOMAS IN MICE

Studies have indicated that growing regions of an organism are electronegatively charged with respect to the non-growing

parts. Growing human uterine tumors, for example, will cause the uterus to be electronegative with respect to the outer abdominal surface. In some laboratory animals, the tumor itself is negatively charged. It would appear, therefore, that application of low voltage electrical currents might alter the growth rate of tumors.

Investigators at the Applied Physics Laboratory of the Johns Hopkins University conducted a series of preliminary experiments to test this hypothesis.

Their procedure consisted of placing a copper or zinc electrode on the unbroken skin over the tumor area in mice with transplanted

sarcoma-180. The second electrode consisted of a saline-saturated sponge rubber rest to which the animal was attached, contact being made over an unclipped ventral area. Control animals were positioned in the same manner but did not receive any electrical current. For tumor inhibition studies, the cathode was placed on the tumor area, and for acceleration studies, the polarity was reversed.

Dr. Carroll E. Humphrey reported that within 21 days all control animals had died. In the group of mice treated for tumor inhibition, however, 60 percent of the tumors had shrunk, hardened, and dropped off, leaving a new skin surface at the site. No significant increase in tumor size was noted in any of the animals in the acceleration group.

In another experiment carried out to confirm these original findings, complete tumor regression occurred after 24 days in seven out of 18 treated mice.

Dr. Humphrey concluded that the results were sufficiently encouraging to warrant continuation of these studies with other types of tumors.

SURVIVAL OF CANCER PATIENTS

SURVIVAL RATE OF BREAST CANCER PATIENTS UNCHANGED OVER PAST TWENTY YEARS

The survival rate of patients with breast cancer, the leading cause of death due to cancer among women, has remained

stable for a quarter of a century. This conclusion was confirmed by a statistical study of data obtained in the State of Connecticut, in which the 10-year survival rate was 38 percent for cases diagnosed during 1935-44 and 39 percent during 1945-54.

The survival experience of some 10,000 patients with primary cancer of the breast diagnosed in Connecticut in the 20-year period from 1935 to 1954 was studied in detail by scientists of the Biometry Branch who collaborated with investigators of the Connecticut State Department of Health. Survival of patients was compared with the expected survival experience for a group of women from the general population, and relative survival rates were computed as the ratio of observed to expected survival rates.

Sidney J. Cutler reported that the relative survival rate for the first 5 years during both periods, 1935-44 and 1945-54, was approximately 50 percent. This indicates that, based on survival expected in the general population, one-half of the

patients had died by the end of the fifth year. Furthermore, patients alive 10 years after diagnosis of cancer were not free of an excessive mortality risk, and even after 20 years of follow-up, the rate of mortality in the population of breast-cancer patients exceeded the rate in the general population.

Other results showed that 1) of patients with localized tumors alive at the end of the tenth year, only 82 percent of the expected number of survivors were alive at the end of the twentieth year; and 2) the most favorable survival experience was observed among patients with localized tumors treated by surgery only. Yet, in this group, mortality was greater than expected for 15 years after diagnosis of the disease and the mortality rate did not equal that in the general population until the 16th year.

The results obtained in Connecticut were comparable with those reported from leading treatment centers, such as the Mayo Clinic and Johns Hopkins Hospital. Since incidence, mortality, and survival rates have been stable for a good many years, Mr. Cutler concludes that "future improvement is more likely to result from the development of new therapeutic techniques rather than from further refinement of current methods."

MEETINGS ON INTERNATIONAL COOPERATION IN EVALUATING SURVIVAL EXPERIENCE HELD

The End Results Evaluation
Section of the Cancer
Chemotherapy National Service
Center held two conferences

on international cooperation in the evaluation of end results in the treatment of cancer. Scientists from England, Denmark, Finland, France, Norway, Russia, and the United States met with members of the National Cancer Institute staff to discuss plans for national, collaborative studies on end results, epidemiology, and incidence of cancer in various population groups throughout the world.

Conference participants discussed uniform procedures and definitions of terms to be used in reporting survival experience of cancer patients in the various countries. The possibility of carrying out collaborative studies on the epidemiology of cancer, comparing incidence in various population groups and under differing environmental conditions, was discussed.

The conferences were an outgrowth of the National Cancer Institute's End Results Evaluation Program in which more than 200 United States hospitals are cooperating. The goal of this research effort is to permit meaningful evaluation of end results in cancer by collecting and analyzing information

diagnosis, methods of treatment, and survival of cancer patients in this country. There is particular interest in comparing the results of accepted methods of treatment, such as surgery and radiation, with those obtained in the use of chemotherapeutic and hormonal agents.

Cooperating hospitals annually submit specified data on individual cancer patients, identified by case number only, to the National Cancer Institute for analysis. This information will be evaluated and compared with that obtained from other countries in an effort to point up the efficacy of various methods of cancer treatment.

Development is under way for a program of international comparisons of end results in cancer for the Eighth International Cancer Conference to be held in Moscow in the summer of 1962. Such comparisons should allow evaluation of therapy so that methods found superior in one country could be more rapidly adopted in other regions.

Dr. Michael B. Shimkin, Chief of the Biometry Branch and Assistant Chief for Clinical Activities of the Service Center, was chairman of the first conference, held in Bethesda in January, and Dr. J. Clemmesen of Denmark was chairman of the second one held in October.

SPECIAL TRAINING

SENIOR MEDICAL SCHOOL
STUDENTS BENEFIT FROM
SPECIAL CANCER COURSES

Drs. Donald R. Green, Emory
University, Atlanta, and
Leonard W. Towner, Long Beach
State College, Long Beach,

California, studied the effectiveness of special courses given by a number of medical schools to improve the teaching of cancer.

Results of a 150-item examination in the subject matter of cancer administered annually to medical students provided the criterion of knowledge about cancer. The test scores were correlated with the number of hours spent by the students in 1) lectures--listening to others talk about cancer; 2) seminars or discussions--talking with others about cancer; 3) pathology laboratories--working with tumors but not patients; 4) clinics and wards run by doctors exclusively--watching others work with patients; and 5) clinics and wards where the students participated directly--working with patients. Analysis was made of the results obtained from 28 medical schools. Particular attention was given to senior-class results, since it is the knowledge demonstrated by the senior that is of most concern.

At the senior level, seminars and discussions were most effective, especially along with working with patients; at the junior level, laboratory work and work with patients contributed significantly to cancer knowledge. The results for the sophomores and freshmen were inconclusive.

The investigators conclude that the special courses in cancer helped senior medical students organize and integrate the knowledge previously acquired in lectures and course work, particularly if the special teaching methods required the active participation of the students.

INSTITUTE CONDUCTS SECOND LABORATORY DEMONSTRATION CONFERENCE FOR TEACHERS

The second laboratory demonstration conference for high school science teachers was conducted by the National

Cancer Institute in October. It was organized by a committee representing the National Cancer Institute, the National Science Teachers Association, and the Office of Education, under the leadership of James F. Kieley, Information Officer, National Cancer Institute. The first conference was held in 1955.

Over 200 teachers from the District of Columbia, Maryland, Virginia, and Delaware observed the demonstrations especially designed by National Cancer Institute research scientists to suggest source material for classroom lessons. After the demonstrations, they heard a discussion on classroom usefulness of the material by a panel consisting of Dr. John W. Renner, Deputy Executive Secretary of the National Science Teachers Association, Washington; Dr. Zachariah Subarsky of the Bronx High School, New York; and Dr. Ruth E. Cornell, Chairman, Secondary Science Department, Public Schools, Wilmington, Delaware.

Additional demonstrations were arranged by the National Institute of Arthritis and Metabolic Diseases.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

HEART DISEASE

1959

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Heart Institute

ATHEROSCLEROSIS AND CORONARY HEART DISEASE

ADRENAL OVERACTIVITY MAY
ACCOUNT FOR RISING BLOOD
LIPIDS IN PSYCHIC STRESS

Experimental observations in
the National Heart Institute's
Metabolism Section provide a
plausible explanation for the

manner in which psychic tension is manifest in changes in the blood lipid pattern. The findings, which are now being extended to humans, implicate the cortical as well as the medullary hormones of the adrenal glands in elevations of blood lipids that occur during emotional stress.

Physicians have long felt that the rapid pace of modern civilization might somehow be contributing to the development of heart disease. The man who develops coronary artery disease is very frequently a hard-driving individual living in a state of more or less constant tension. In recent years evidence has accumulated that one way in which nervous tension may accelerate the development of coronary artery disease is through an elevation of the serum cholesterol level.

Dr. Eleazar Shafrir and Dr. Daniel Steinberg, in the Section on Metabolism, have shown that epinephrine, the adrenomedullary hormone released in stress situations, causes an elevation of both the unesterified fatty acids and the lipoproteins of the serum in dogs and in rats. When, however, the adrenal glands have been removed, epinephrine no longer has these effects. By giving the animals cortisone, the potency of epinephrine in raising serum lipid levels was fully restored. Furthermore, in normal animals, the simultaneous administration of epinephrine and cortisone yielded elevations of serum cholesterol that were two or three times as great as those due to epinephrine alone. These findings, which were presented in part at the 1959 Federation Meetings, appear in the Journal of Lipid Research.

This synergistic action of epinephrine and cortisone is of particular interest since the activities of both the adrenal medulla and the adrenal cortex are known to increase under stress. Consequently, the overactivity of both parts of the adrenal gland during stress creates what may be a particularly "dangerous" hormonal pattern leading to significant elevation of serum cholesterol levels.

These experimental observations, which are being extended in human studies, may thus provide a plausible explanation for the manner in which psychic tension becomes somatically manifest. For example, the studies of Friedman, Rosenman and Carroll on the significant elevations of serum cholesterol levels occurring in tax accountants just prior to April 15th and the studies of Wertlake et al showing a rise in cholesterol levels of students subjected to the stress of examination week may be accounted for, at least in part, on the basis of adrenal overactivity. It will be important to study the possible correlation between hypercholesterolemia and adrenal function more carefully and to evaluate the possible importance of this factor relative to the other factors, such as diet and heredity. Whether the periodic elevations of serum cholesterol that occur during stressful periods are important in the genesis of atherosclerosis remains to be determined. If stress-induced adrenal overactivity should be a significant factor, measures directed at the control of emotional strain may turn out to be an important element in the prevention of atherosclerosis.

UNSATURATION OF DIETARY FATS IMPORTANT BLOOD- LIPID-LOWERING FACTOR

The ability of a dietary fat to reduce abnormally high levels of blood cholesterol and other fatty constituents of the blood appears

to depend upon its total unsaturated fatty acid content rather than the essential fatty acids or sterols it may contain, an NHI grant-aided study indicates.

Findings from the study were reported in the British journal Lancet by the eight investigators: Drs. Edward H. Ahrens, Jr., Jules Hirsch, Malcolm L. Peterson, William Insull, Jr., Wilhelm Stoffel, and John W. Farquhar of the Rockefeller Institute, New York; Theodore Miller of Marine Chemurgics, Inc., Morehead City, North Carolina; and Dr. H. J. Thomasson of the Unilever Research Laboratories, Vlaardingen, Holland.

Two patients, one with hyperlipemia and the other with hypercholesterolemia, were studied continuously for 21 and 22 weeks on a metabolic ward. They were given special diets in which corn oil or menhaden fish oil was the sole dietary fat in three successive feeding periods. Corn oil, highly unsaturated and rich in essential fatty acids and sterols, was given during the first

and third periods. Menhaden oil, also highly unsaturated, but, unlike corn oil, poor in essential fatty acids and sterols, was substituted for the corn oil, calorie for calorie, during the second period.

The menhaden oil was carefully processed especially for this study from 500 pounds of freshly-netted menhaden--an Atlantic fish used for fertilizer, oil, and fish meal for pet food. The essential fatty acid composition, less than 4 percent of the total fatty acids, was accurately determined by using two different analytical methods--gas chromatography and bioassay in animals. The marine oil also contained "negligible amounts" of sterols.

The investigators report that, as expected, corn oil produced a striking decrease in all serum lipid levels--cholesterol, phospholipids, and triglycerides--during the first experimental period. The initial serum lipid responses to corn oil were maintained or further lowered by substitution of menhaden oil in the second period. During the third period, corn oil produced slight serum lipid elevations in one patient and negligible changes in the other patient.

Although serum lipid levels remained comparably low throughout the experimental period, the fatty acid composition of serum lipids was altered with each change in dietary fat.

The investigators conclude from their findings that "The effects on serum lipid levels were unrelated to the essential fatty acid and sterol contents of the dietary fats under consideration."

BLOOD CLOTTING TENDENCY AS FACTOR IN HEART ATTACKS EMPHASIZED

The importance of blood clotting tendencies, as distinguished from atherosclerotic lesions, in the genesis of coronary heart attacks receives emphasis from a comparison by St. Louis workers of autopsy records of Uganda (East Africa) and St. Louis population groups. A low incidence of myocardial infarction in Uganda Negroes was found by the St. Louis investigators to be associated with a similarly low incidence of occlusive blood clots (thrombo-embolic phenomena) in the venous system, where atherosclerosis does not develop. And a high incidence of clots in the veins of the St. Louis Negro and White subjects was found associated with their known high incidence of coronary heart attacks. The correlation found suggests the possibility that a common factor affecting blood clotting or clot dissolution is operating in both the venous and arterial occlusions.

Dr. Wilbur A. Thomas while at Washington University School of Medicine, St. Louis, and his co-workers there have for some

time held the view that disorders of blood clotting or clot lysis may be of major importance in the causation of myocardial infarction. (Dr. Thomas is now professor of pathology at Albany Medical College.) Unfortunately for any direct determination of the role of clotting in coronary occlusion, it is presently impossible to distinguish the contribution of the clot from that of the fat and other material which occur together in the occlusive arterial lesion.

It occurred to the St. Louis workers, however, that studying thrombotic phenomena in the low pressure venous and pulmonary circulation, where atherosclerosis **does** not occur, offers an indirect approach to answering this question. They reasoned that if incidences of occlusive blood clots in lungs and veins correspond to the widely differing incidences of myocardial infarction that occur in different population groups, abnormalities of clotting or clot lysis can probably be blamed, at least in part, for the cardiac picture.

They chose groups of Uganda Negroes and St. Louis Negroes and Whites for their geographic and racial study of this relationship because previous autopsy studies had reported the incidence of myocardial infarction to be high in St. Louis Whites, low in Uganda Negroes, and intermediate in St. Louis Negroes.

With the aid of NHI grant support, Dr. Thomas and Dr. A. A. Dimakulangen studied the autopsy records of Washington University and Makerere Medical College (Uganda) for the years 1951 to 1956 on all cases age 40 and over. In a two-way age-sex matched comparison of 634 cases, they found a 21.5% incidence of myocardial infarction and a 21.5% incidence of pulmonary-venous thrombotic phenomena among the St. Louis Whites, while the Uganda Negroes showed a 0.2% incidence of the cardiac and a 2.4% incidence of pulmonic-venous clots.

In a three-way age-sex matched comparison of 206 cases, St. Louis Negroes were found to have an 18.9% incidence of the cardiac and 23.8% of the pulmonic-venous phenomena, while the Uganda Negroes had 0.5% cardiac and 5.3% of the pulmonic-venous phenomena.

Although the difference between the incidences in the St. Louis Negroes and Whites was not statistically impressive, the differences between the Uganda Negroes and either the St. Louis Negroes or the St. Louis Whites were highly significant.

"These figures suggest that the low incidence of myocardial infarcts among Uganda Negroes is perhaps due in part to a lesser tendency of their blood to clot (or clots to lyse) than that of St. Louis Whites or Negroes," the investigators

reported at the meeting of the Federation of American Societies for Experimental Biology.

DIRECT SURGICAL ATTACK ON OCCLUDED CORONARIES FOUND PAIN-RELIEVING BUT RISKY

One surgical method of treating coronary atherosclerosis involves direct attack on the diseased artery lining, using special dis-

secting loops or cutting probes to remove the occluding core of the artery and restore blood flow to the heart muscle. Recent studies at the University of California suggest that such operations may greatly relieve pain and increase exercise tolerance, but may carry considerable risk.

In 1957 Dr. Angelo May of San Francisco reported successful use of a specially constructed cutting needle-probe to "ream out" the main coronary branches in dogs. The same month (June, 1957), Philadelphia surgeons Charles P. Bailey and William Lemmon, together with May, reported using May's technique in two patients, one of whom was reported strikingly improved by the operation.

Working since 1954 under an NIH research grant, surgeons Jack Cannon and William Longmire of the University of California Medical Center have been investigating coronary endarterectomy along four avenues.

The first aspect of their approach involved postmortem dissection of human hearts to learn whether it is feasible to dissect out the coronary linings. They found that the plugged segments tend to appear first in the big outer branches of the coronaries, where they are accessible to the specially sharpened stripping loops they designed for the purpose, and that the intimal lining, which collects the troublesome fatty material, can easily be separated from the muscular medial layer.

Encouraged by such findings, they moved to the second phase--the development of a sound technique of coronary endarterectomy. This they practiced in dogs, in which they first produced coronary occlusions by inserting a length of plastic tubing into the artery. Despite the difficulty of working inside these small vessels, which move with the beating heart, they were able to remove the coronary lining and restore blood flow in significant numbers of dogs.

In the third aspect of the study--the establishing of criteria for selecting patients--the investigators imposed three basic restrictions: (1) the patient had to be completely incapacitated by the disease and willing to accept almost any gamble, (2) other less drastic methods must have been tried and failed,

(3) the diagnosis of obliterative coronary disease had to be absolutely certain.

"We wish to emphasize no patient has been 'sold' this operation," Dr. Cannon explains. "It was offered him. The ins and outs were discussed and he was told his chances of coming through were not even 50-50. Such patients were always given time to think the matter over. No urging of any sort was practiced."

In the fourth phase of the study--clinical experience--nine patients, all with intractable angina pain, underwent surgery. One died on the operating table before the coronary procedure could be undertaken, and two others died during operation. A fourth, markedly relieved of his angina after surgery on his left coronary, developed angina again four days later and subsequently died. The other five survived and were improved.

"The most dramatic improvement in the survivors was attained in a patient who had endarterectomy of the right main coronary alone," the investigators write. "He had been totally incapacitated and was waiting to die preoperatively, but since operation has not experienced pain or exercise electrocardiographic changes in response to any amount of exercise, and he has resumed full time employment. . . In all survivors the repeat exercise electrocardiogram has shown either marked improvement or become normal."

A report of this work has been published by Drs. Cannon, Longmire, and Dr. Albert A. Kattus in the journal Surgery.

**SERUM TRIGLYCERIDES THOUGHT
MORE INDICATIVE OF CORONARY
RISK THAN CHOLESTEROL LEVEL**

Heart Institute grantees in New Haven report evidence that elevations of serum triglyceride concentration are more definitive

of coronary disease than are elevations of cholesterol or phospholipids, the other major lipid fractions.

"Although triglycerides rather than cholesterol are chiefly responsible for turbidity of serum and constitute an important fraction of beta lipoproteins, the quantitative estimation of triglycerides has been largely neglected in studies of serum lipids in coronary artery disease," the Yale scientists point out.

"The present research was undertaken to observe the interrelations of the three major serum lipid fractions (cholesterol and its esters, phospholipids, and triglycerides). . . The neglected fraction appeared to be most frequently elevated in coronary disease."

The investigators, Drs. Margaret J. Albrink and Evelyn B. Man, report in the AMA Archives of Internal Medicine that the triglyceride, or "neutral fat", component was elevated above a normal level of 5.5 milli-equivalents per liter of serum in 85% to 90% of the 82 coronary patients in the population studied.

The 5.5 figure was designated by the investigators (on the basis of their data from the lipid analyses in the coronary patients and 134 healthy normal men) as the triglyceride level best dividing the coronary from the normal populations in the study. This figure was only slightly below the upper limit of normal (5.9 mEq. per liter), the investigators explain. Both the 5.5 and the 5.9 figures were used throughout the report for comparison of the different study populations.

The triglyceride concentrations for 70% of the coronary population were reported by the Yale workers to fall above the 5.9 figure, while those of almost the entire normal population fell below this figure.

In contrast, only 18% of the coronary patients were found to have cholesterol concentrations above the upper limit of normal designated for this lipid (269 mg.%). Furthermore, the investigators point out, decade by decade, the difference in cholesterol concentration between coronary and normal subjects was not significant except in the fourth decade.

"The present report suggests that an error in the metabolism of triglycerides is the lipid abnormality operative in coronary artery disease," Drs. Albrink and Man summarize.

REDUCED FIBRINOLYSIS AFTER FATTY MEALS RELATED TO CHYLOMICRON LIPOPROTEINS

The lessening of normal clot-dissolving activity in human blood that occurs after fatty meals has been traced by San Francisco workers to the chylomicrons, lipoprotein particles in which absorbed dietary fats are transported from the intestine into the blood stream.

The blood of normal fasting individuals has been seen by different investigators to dissolve blood clots under a variety of experimental conditions. This normal clot-dissolving, or "fibrinolytic", activity, in balance with the constant tendency for clots to form in inflammatory reactions, is regarded by many authorities as an important normal repair mechanism, operative throughout the organism in health and disease.

Fibrinolytic phenomena are of increasing research interest, partly because of the possibility that disorders of the fibrinolytic mechanism may be involved in atherosclerosis, and partly

because of recent promising developments in the fibrinolytic treatment of clot-obstructed blood vessels.

In 1956, H. B. W. Greig of the South African Institute of Medical Research reported that fatty foods can interfere with the fibrinolytic activity of blood. This reported inhibition of fibrinolysis by dietary fats has been confirmed, with the aid of NHI grants, by Drs. Thomas C. Merigan, John W. Farquhar, James H. Williams, and Maurice Sokolow of the University of California School of Medicine.

They report in Circulation Research that meals of cream consistently interfered with the clot-dissolving activity of blood plasma from five normal subjects. The fibrinolytic activity of plasma measured during fasting was found to be reduced an average of 61% when measured again four hours after a test meal containing 1.5 grams of butterfat per kilogram of body weight.

These workers also set out to learn which of the known forms of fatty substances (lipids) appearing in the blood after fatty meals are responsible for inhibiting the clot-dissolving mechanism. "Since the change in plasma lipids after ingestion of fat consists primarily in the appearance in the blood of chylomicrons, it seemed appropriate to study first the effect of this fraction on the fibrinolytic process," the San Francisco workers explain. This they did by contrasting the fibrinolytic activity of a plasma sample from which the chylomicrons had been removed (plasma fraction II) with a sample from which chylomicrons had been removed and then re-suspended (fraction I). In eight cream-feeding experiments using six normal subjects, the fibrinolytic activity of fraction I (plasma milky with re-suspended chylomicrons) was significantly less than that of fraction II (chylomicrons removed by ultracentrifugation).

"Our experiments indicate that a single species of plasma lipoprotein (chylomicrons) is responsible for the observed inhibition," the San Francisco workers report. "Studies now in progress will attempt to define the properties of human chylomicrons responsible for this inhibition."

"STRESS" AND HEIGHTENED ADRENAL ACTIVITY RESULT IN HEART ATTACK IN RATS

The success of a Cincinnati research team in producing heart attacks in rats on a normal diet is attributed by them largely to the effects of stress on the adrenal glands. Heavy reproduction in females was the experimental stress stimulus, and its cumulative effect on the adrenals was heightened by injections of adrenal-stimulating pituitary hormones (ACTH) three times a week,

and by the removal of one kidney. Of 100 female breeders thus treated for 5 to 7 weeks, 75 developed coronary arteriosclerosis, and 30 of them had myocardial infarctions.

This NHI grant-supported study is reported in Proceedings of the Society for Experimental Biology and Medicine by Drs. Bernard C. Wexler and Benjamin F. Miller of the Cincinnati Jewish Hospital and the University of Cincinnati.

"It is our supposition that repeated breeding puts stress on the adrenal glands and that this stress is further heightened by administration of repeated doses of ACTH," Wexler and Miller write. "Possibly the unilateral nephrectomy intensifies the action of ACTH."

With the announcement of their "stress" method, two widely differing methods become available for producing "model" heart attacks in rats. The first method, announced earlier (in 1957) by Hartroft and Thomas of St. Louis, is described as dietary. In line with its authors' view that abnormal tendencies to clot formation may be of primary importance in atherosclerosis, this dietary method involves feeding one of the saturated fats (butter, lard, or hydrogenated vegetable oil) that raise the blood cholesterol and are believed to interfere with normal clot-dissolving (fibrinolytic) mechanisms, and employs other techniques for raising the blood lipids. The dietary method causes myocardial infarction by plugging the rat coronaries with mixed clot and fatty material, according to the reports of Hartroft and Thomas.

Unlike this dietary method, the "stress" method of Wexler and Miller produces extreme swelling and thickening of the middle (medial) and inner (intimal) arterial layers sufficient of itself to interfere with blood flow. The deposition of occlusive clot and other material in the artery, and myocardial infarction, often result, but this stress method involves no excess dietary fat or salt, does not increase blood cholesterol, and little or no fatty material is deposited in the heart-damaging occlusive lesions it produces. These investigators call attention to the clots in emphasizing similarities between the rat disease and human arteriosclerosis. They also mention differences in the susceptibility of different rat strains to the "stress" treatment in terms of hereditary variations of human susceptibility.

The rat disease they describe differs, however, from the occlusive coronary disease most frequently reported by pathologists from human autopsy material and from that produced in animals by most experimenters, chiefly in the lack of fatty depositions. "Only an occasional lesion, and usually an advanced one, showed demonstrable fat," they report. The extreme swelling within

the coronary tissues of the rat, sufficient of itself to severely limit the blood passageway, also differs from the atherosclerosis most frequently reported in human arteries. Thus the form of "stress" arteriosclerosis produced in these rats was varied, as the investigators point out in their summary, and reflected differences from, as well as similarities to, the lesion usually reported in human atherosclerosis.

**PLASMIN INFUSIONS DISSOLVE
CORONARY-OCCLUDING CLOTS
INDUCED IN TEST ANIMALS**

Experimental work at the Sloan-Kettering Division of Cornell University Medical College suggests it may be possible to

dissolve away human coronary occlusions by infusions of concentrated plasmin, a clot-dissolving enzyme found normally in the blood. The animal experiments appear to prove the dissolution of coronary clots by plasmin within 4 to 6 hours after their formation.

A technique was first developed for producing coronary clots in dogs which resemble those observed in heart attacks in man and which could be photographed serially in the living animals by injecting their coronary systems with substances opaque to X-ray. "Bulging intra-coronary clots" of measurable dimensions were produced by injecting a clot-forming mixture of blood and serum into a segment of a main coronary artery which had been temporarily tied off. This was accomplished in 16 animals.

In eight of these subjects, an infusion of plasmin (4000 units per kilogram per hour) was started from one to eight hours after clot formation. Complete dissolution of the coronary thrombi with restoration of blood flow through the plugged coronary artery, was demonstrated by X-ray photographs within 3 to 7 hours after occlusion in four of the plasmin-treated subjects, the New York workers report in Circulation. Partial dissolution (60% or more) was recorded from the other four experimental dogs.

The eight control dogs, which had not received plasmin, were autopsied 7 to 15 hours after their coronary occlusion. The thrombus had persisted in seven. In the eighth control dog, the clot dissolved spontaneously seven hours after its formation.

The microscopic appearance of the hearts of the treated dogs was striking in contrast to those of the untreated, the Cornell investigators report. The treated hearts showed less edema, capillary dilatation, and deposition of clot material (fibrin) over the surface of the myocardial infarct. The tiny clots (microthrombi), commonly seen in the capillaries of the infarcted heart muscle in the control group, were not seen at all in the treated hearts, even those which were examined

before the occluding clot in the main coronary had dissolved. "These observations suggest that fibrinolytic blood may affect not only the initial occluding thrombus, but penetrate into the infarct itself by collateral perfusion," the investigators comment.

"Since no deleterious effects of plasmin, such as myocardial rupture or intramyocardial hemorrhage were observed," they summarize, "we think that our experimental evidence offers a sound rationale for a trial of fibrinolytic therapy of human myocardial infarction. With the recent development of purified preparations of plasmin for human use, such a study is now possible."

Participating in this NHI grant-supported study were Drs. Paul Ruegsegger, Irwin Nydick, Robert Hutter, Alvin Freiman, Nils Band, Eugene Clifton, and John LaDue.

LINOLEATE, MAJOR DIETARY FATTY ACID, AS SOURCE OF FOOD ODORS AND FLAVORS

Chemical studies at Pennsylvania State University suggest that the characteristic "deep fried" flavor that results when fats are subjected to intense and prolonged moist heat, as in baking, broiling, and frying, derives to a large extent from decadienal, a compound resulting from the oxidation of linoleate. Linoleate, an unsaturated fatty acid of international research interest in terms of atherosclerosis, is abundant in most dietary fats that have not been hydrogenated during processing.

During chemical studies of substances evaporating from major food fats under sustained moist heat, investigators in the Department of Dairy Science of Pennsylvania State University were impressed by the predominance and the odor of decadienal in the condensed vapors. Cottonseed oil, soybean oil, beef tallow, and lard, subjected to 438° F. steam heat for 3 to 5 hours, yielded distillates strongly reminiscent of fats used repeatedly in frying foods, the investigators observed.

These chemists, Stuart Patton, Isabel Barnes, and Laura Evans, analyzed the distillate and traced the "deep fried" odor to decadienal, a product of heat and moisture which they describe as a flavor compound of "fantastic" potency, ranking in flavor intensity with the very powerful sulfur compounds. Their data, published in The Journal of the American Oil Chemists Society, and other observations in the literature of oil chemistry indicate that decadienal results from the decomposition of linoleate in most or all common edible fats and that high temperatures, as in baking, broiling and prolonged and repeated frying, favor its formation.

In tests of its flavor potency, taste observers were able to detect decadienal in dilutions as weak as 1/2 part of the

compound per billion parts of water. Recognizing the flavor, the test subjects commonly used the terms "deep fried," "restaurant," and "oily" to describe it, the chemists report. "Flavor and odor qualities of the dienal perhaps are described best by the term 'deep fried'," they suggest.

The investigators had several reasons for suspecting that linoleate oxidation is the source of the flavor compound. Other chemists had pointed out that linoleate, one of the most unsaturated of the common dietary fatty acids, is one of the most readily oxidized. It is also one of the most abundant fatty acids in most food fats that have not been saturated by chemical hydrogenation. From their own analytical studies of the four natural food fats, it appeared that the amount of decadienal resulting from the moist heat was generally related to the amount of linoleate in the fat.

Confirmation was obtained when they subjected linoleate alone to 400°-480° F. of steam heat. The strong "deep fried" odor of decadienal resulted, and the predominance of the decadienal in the distillate, as measured analytically, established linoleate as the source of the flavor compound in the four fats.

"The intense flavor properties of the compound coupled with its potential of formation from the major edible fats, or more generally from any fat containing linoleate, suggest that it is a very commonplace component in food odors and flavors," the investigators write. "More particularly it is postulated that decadienal contributes to the flavor and aroma of foods in which the fat comes in contact with moisture at relatively high temperatures. The classic example is deep fat frying, but broiling, frying, and baking also fit the definition under certain conditions. The baking of pie crust is probably a good example."

The Heart Institute is supporting a number of studies, including this one, concerned with the dietary significance of fatty acids. Linoleate is of particular interest because of its unusual effectiveness in lowering the blood cholesterol level and because vegetable oils of which linoleate is the chief fatty acid constituent (corn oil, cottonseed oil, safflower oil and others) are coming into increasing use by physicians in the treatment of patients with atherosclerosis and coronary disease.

Although leading authorities now agree that the blood cholesterol of most persons can be lowered by the judicious substitution of unsaturated for saturated fats in the diet, there is little agreement on the advisability of recommending such changes, even for patients. The disagreement derives from a number of unresolved issues, including the lack of clear proof that lowering

the blood cholesterol would help prevent or reverse the artery-clogging disease.

But the controversy also reflects differing views on the question of whether the substitution of unsaturated fats would necessitate sacrifices of food palatability and changes in patterns and habits of processing and preparation too drastic to be acceptable.

The report of Patton, Burns and Evans contains no suggestions or comments as to whether the linoleate content of foods or its tendency to oxidize and form decadienal in them is either "good" or "bad" from a health or any other standpoint. Data from analytical studies such as theirs, however, may ultimately help provide a more objective basis for industrial and medical interests to judge the usability and acceptability of fats and fat constituents which, though not customarily used in food preparations, may have health advantages.

BLOOD PRESSURE LOWERING
DRUG, JB 516, ALSO FOUND
TO RELIEVE ANGINA PAIN

JB 516, a potent new MAO inhibitor which lowers high blood pressure, has been reported by NHI grantees in Los Angeles as also effective

against the chronic chest pain of coronary artery disease. Drs. Rexford Kennamer and Myron Prinzmetal of Cedars of Lebanon Hospital and the University of California School of Medicine report the study in The American Journal of Cardiology. Cautioning that their study in 31 patients was preliminary and limited in scope, they report that JB 516 appears from the findings to be of "great value" as an anti-anginal agent, combining pain relief with low toxicity.

Some of the 31 patients had been successfully treated for nine months. "Marked improvement" (the patient had no further pain) was reported in 12, "moderate" (notable improvement, but use of nitroglycerine still necessary) in 11, and questionable or no improvement in 8 of the 31 patients.

Jitteriness (2 patients) and insomnia (4 patients) were among the side effects, for JB 516 is a powerful central nervous system stimulant. Postural hypotension, noted in 5 patients is also not considered surprising in view of the known effectiveness of the drug in lowering blood pressure. The Los Angeles team found no serious toxic reactions, and the most troublesome side effects, insomnia and low blood pressure, were controlled through dosage regulation in most instances.

JB 516 was first synthesized by Dr. John H. Biel, a chemist with Lakeside Laboratories, Milwaukee, Wisconsin. The drug, a hydrazine analogue of amphetamine, is an unusually potent member of a large and diverse category of drugs which have in common the fact that they inhibit the enzyme, monoamine oxidase (MAO), in the tissues. JB 516 is one of the MAO inhibitors which have also been reported effective as "psychic energizers", particularly in the field of psychopharmacology.

Following initial chemical and pharmacological studies at Lakeside, more detailed pharmacologic work was conducted in the laboratories of Drs. Sidney Udenfriend and Bernard B. Brodie, NHI. JB 516 became known in 1958 as the first MAO inhibitor to be used successfully in the treatment of human hypertension. Reported by Drs. Luther L. Terry, Albert Sjoerdsma, and Louis Gillespie of NHI, this advance marked the introduction of MAO inhibition as a promising new approach to both the study and control of human cardiovascular disease.

Very recent evidence from the NHI studies of a seriously limiting side effect for JB 516 (possible impairment of vision at continued high dose levels) does not negate the value of this advance, for other enzyme inhibitors with widely varying effects are being discovered and synthesized in increasing numbers and variety, as interest grows among biochemists and pharmacologists in enzyme inhibition as a basis for drug development.

HEART IRRADIATION INCREASES SURVIVAL OF DOGS FOLLOWING CORONARY OCCLUSION

X-rays, applied to the heart in successive, small doses over a period of 2 to 2½ weeks, conferred considerable protection

against the ordinarily disastrous consequences of tying-off a major coronary artery, report Drs. Elliot Senderoff, David J. Kavee, Richard J. R. Johnson, and Ivan D. Baronofsky, from dog studies conducted at Mt. Sinai Hospital, New York City. The studies, aided by a National Heart Institute grant, constitute a unique, non-surgical approach to the problem of increasing the supply of blood to the heart muscle. Results of the investigation appear in the Proceedings of the Society for Experimental Biology and Medicine.

On the premise that proper doses of radiation would increase the heart's blood supply by dilating existing blood capillaries, the Mt. Sinai researchers treated the hearts of 33 dogs, each with a total of 1300 or 2000 Roentgens of radiation applied in five small doses through the chest. A group of 23 non-irradiated animals served as controls.

Protective effects of the X-ray treatments were revealed when, two to six days later, a major branch of the left coronary artery was tied-off in the experimental and control animals and 24-hour survival rates compared. The two groups of animals were each further divided into two subgroups to compare the effects on survival of using techniques for defibrillating, or restoring normal heart beat, in those hearts which had developed a usually fatal, abnormal heart rhythm.

Defibrillation procedures--heart massage, electrical shocks, and drug injections--averted fatal results in only 33 percent (6 of 18) of the subgroup of non-irradiated dogs on which these were tried following coronary occlusion. Five control animals in which defibrillation was not attempted died within 24 hours. In contrast, 47 percent (7 of 15) of the irradiated dogs in the subgroup not subjected to defibrillation survived the coronary occlusion. Twenty-four hour survival rates soared to 83 percent (15 of 18) in the subgroup of irradiated dogs in which defibrillation was used, and only 7 of these required defibrillation. The workers conclude that "ability to defibrillate radiated dogs is strikingly evident."

The investigators believe that increased survival of the X-ray treated dogs following coronary occlusion is the result of back flow of blood from adjacent coronary vessels. More detailed studies of the effects of cardiac irradiation are in progress.

IF USE REQUIREMENTS ARE MET, STREPTOKINASE IS EFFECTIVE IN HUMAN OCCLUSIVE DISEASE	Proof that the bacterial enzyme streptokinase can be used to remove clots blocking human blood vessels is reported from
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NHI-supported studies of experimentally induced clots in the veins of human volunteers.

The investigators, Drs. Alan Johnson and Ross McCarty of New York University, have published their findings in the Journal of Clinical Investigation. The studies indicate that streptokinase must be infused into the blood under sharply defined biochemical conditions for optimal effectiveness in treating blood vessel occlusions in man.

The investigators report that it is desirable to be aware, during the treatment, of the patient's blood levels of streptokinase, plasminogen, plasmin, and prothrombin time, as well as other blood factors. Plasmin is the blood enzyme that normally dissolves fibrin and thus helps to maintain the normal fluid state of the blood. Plasminogen represents the precursor system that produces the plasmin normally. The drug streptokinase stimulates the conversion of plasminogen to plasmin. The amounts of the components in the blood vary greatly from patient

to patient and time to time as does the level of streptokinase antibody, which must be neutralized before streptokinase treatment is fully effective.

The development of more concise biochemical methods than those presently available for measuring these components is necessary if the maximum benefit of streptokinase fibrinolysis is to be fully realized in the treatment and prevention of human occlusive diseases, the investigators state.

Thirty-eight blood clots 2 to 5 inches long were induced in superficial arm and leg veins in 26 human volunteers by irritating the vessel lining with a tiny barbed wire (dental broach) inserted through a hollow needle. Objective comparison of the effectiveness of three different ways of treating such clots with streptokinase was thus made possible in human subjects with the use of superficial and readily expendible blood vessels. Due to the wealth of collateral venous circulation, the procedure in no way impaired the venous circulation of the volunteers. And there was no evidence of thromboembolic risks (blood clots cast adrift to lodge elsewhere) or any other kind of risk to the volunteers.

When streptokinase was infused so as to produce large amounts of plasmin but no measureable streptokinase in the circulation (7 instances), there was no persistent clot disappearance, the investigators report. Although some reduction of the clots occurred in 3 instances and the clot disappeared in 1 instance, all the clots reformed again.

When large amounts of streptokinase were maintained in the blood for long periods of time (7 instances), plasmin precursor components in the blood tended to be depleted, so relatively little plasmin formed. Although the clots dissolved at first, they tended to reform and were thereafter resistant to the treatment. This is explained by evidence that the special effectiveness of streptokinase depends on its ability to activate the components that were incorporated into the clot during its formation, i.e., streptokinase makes the clot "dissolve itself". A clot formed in blood lacking the precursor components would lack potentiality for such "self-dissolution", the investigators reason.

In all of 11 instances in which streptokinase was infused so as to maintain moderate amounts of both streptokinase and plasmin precursor components in the blood, the clots dissolved and did not reform.

BLOOD PRESSURE

BLOOD PRESSURE CONTROL AND SODIUM-TO-POTASSIUM RATIO IN CARDIOVASCULAR MUSCLE

Accumulating evidence has tended to indicate that the potassium content of arterial tissues may govern blood pressure, possibly through changes in the state of contraction of the arterial muscle (and hence in the caliber of the arteries). But findings reported by Drs. S. Charles Freed and S. St. George of the Mt. Zion Medical Center, San Francisco, shift the emphasis from potassium to sodium in this mechanism. Their point of view derives from consideration of the ratio of sodium to potassium, rather than of the concentrations of these salts separately.

Judging from their report in the American Journal of Physiology, their line of exploration and reason seems to have been somewhat as follows. Rats, deprived of potassium in their diets, were seen to develop low blood pressure along with the low potassium concentrations in arterial (aortic) tissue. This was in accord with the reports of others. But when the sodium was also measured and the measurements were made in heart as well as arterial tissue, it was seen that potassium depletion caused an increase of sodium in relation to potassium (the sodium/potassium ratio).

Cortisone injections will restore normal blood pressure in potassium-depleted rats, so the scientists injected cortisone to see how this adrenal hormone would influence the salts in the cardiovascular tissues. With the restoration of normal blood pressure, the scientists saw the normal sodium/potassium ratio restored in both heart muscle and arterial (aortic) tissue. This occurred by mobilization of sodium out of these tissues.

In the light of their evidence that cortisone can mobilize sodium from heart and arterial muscle, the San Francisco workers suggest that the peculiar effectiveness of cortisone in some cases of resistant edema might be due to an improvement of circulatory functions rather than to an action of cortisone on the kidney.

NEW KIND OF ORAL DRUG FOR HYPERTENSION PROMISING IN FIRST TRIALS IN PATIENTS

Preliminary clinical trials of guanethidine against hypertension confirm the promising experimental findings made at Ciba, where this new drug was synthesized. Independent

clinical studies made with NHI grant support at the Cleveland Clinic Foundation and Georgetown University, show that a single daily oral dose of guanethidine can safely lower arterial pressure in human renal and essential hypertension to the levels achieved by ganglionic blocking agents. Both the experimental and clinical studies indicate guanethidine has a mode of action unique among the antihypertensives now in clinical use.

Dr. R. A. Maxwell and co-workers at Ciba Pharmaceutical Products, Inc., first observed guanethidine's novel mode of action in a related compound, 4029 SU. In 1958, they reported that a single dose of 4029 SU would lower the blood pressure of dogs with renal and neurogenic hypertension for as long as two weeks without undesirable side effects. More recently the Maxwell group reported a similar action for 5864 SU, or guanethidine, which appears to be a more useful drug clinically.

From the experimental work in cats and dogs, Maxwell postulated a unique mode of action for the new compounds. He suggested that they inhibit the transmission of sympathetic nerve impulses (which constrict arteries and raise blood pressure) from the nerve ending to the muscle (at the "sympathetic nerve terminals"). Although the familiar ganglionic blocking agents (hexamethonium, pentamethonium) and surgical sympathectomy also work by blocking sympathetic impulses, they work at the level of larger nerve centers (autonomic ganglia). Parasympathetic blockade is blamed for the side effects that characterize conventional ganglionic blocking agents (sexual impotence, fast pulse, constipation, etc.)

It was the apparent absence of parasympathetic blockage in guanethidine, suggesting a novel mode of action, which aroused the interest of clinicians at the Cleveland Clinic Foundation, Georgetown University, and elsewhere.

Their clinical studies suggest that parasympathetic overactivity, rather than blockade, may result from guanethidine therapy. For example, diarrhea and slowed pulse, rather than constipation and fast pulse, were reported as conspicuous side effects of guanethidine by both the Cleveland group (Drs. Irvine H. Page and Harriet P. Dustan) and the District of Columbia group (Drs. Edward D. Frohlich and Edward D. Freis). Disturbance of sexual function, noted in some male patients by the D. C. group, was less complete and therefore less objectionable to the patients than the

impotence characteristically produced by ganglionic blocking agents. No toxic actions were seen in patients studied by either research group, and the side effects of parasympathetic predominance (diarrhea, nasal stuffiness, slow pulse) were not serious.

Careful individual regulation of guanethidine dosage was found necessary to avoid the wide swings in blood pressure characteristic of agents that produce the orthostatic, or "postural" type of low blood pressure. Commenting on this disadvantage in Medical Annals of the District of Columbia, Drs. Frohlich and Freis suggest the use of chlorothiazide as an adjunct to guanethidine to help stabilize the hypotensive response.

"The response was characterized by a potent, orthostatic, antihypertensive effect similar to that seen with the ganglionic blocking drugs but without the side effects of parasympathetic blockade", the District of Columbia workers summarize from their studies in fifteen male hypertensives.

"Treatment of 18 hypertensive patients with guanethidine shows it to be an effective hypotensive agent, with mild diarrhea as the only side effect so far noted," Dr. Page's group reported in The Journal of the American Medical Association. "The fall in arterial pressure is slow and prolonged, often with postural hypotension preceding normal supine pressure. Bradycardia commonly occurs."

This group of compounds is being studied also by the Section of Experimental Therapeutics, NHI.

ARTERIAL HYPOTENSION REFLEX RESPONSE TO STRETCH OF LUNG

A blood-vessel-dilating reflex, often producing marked and sustained lowering of arterial blood pressure, was found by

Los Angeles investigators to result from stretching of the dog lung by either manual traction or positive pressure breathing. This stretch reflex is mediated by the vagus nerve, since it is abolished when this great moderating nerve from the central autonomic nervous system is cut. The findings emphasize the hazard of increasing the airway pressure when positive pressure is used to maintain the breathing of patients with poor circulation.

Increased airway pressure has often been observed to lower blood pressure and heart rate. The reflex mechanism involved has been difficult to demonstrate, however, because of the complex interaction of the nervous and mechanical effects of inflating the lungs.

"Relevant information can be derived only from experiments in which mechanical factors, such as changes of the pulmonary blood flow and tamponade of the heart and great vessels, are separated from nervous factors," Dr. Peter Salisbury and his co-workers at Cedars of Lebanon Hospital, Los Angeles, point out in a report on their NHI grant-supported studies published in Circulation Research.

These workers solved this problem by conducting lung-stretching experiments in animals in which the general circulation was maintained by a heart-lung machine while a separate pump-reservoir maintained the circuit through the lungs. The constant-flow perfusion through the separate circuits thus obtained, and the use of cardiac arrest, assured that the changes recorded in arterial blood pressure were caused by changes in peripheral blood vessel tone or caliber.

In more than 60 experiments of this sort, the systemic arterial pressure consistently fell on lung inflation, the Los Angeles workers report. The same drop in arterial blood pressure was obtained by manually squeezing and stretching lung lobes.

If such stimuli were applied when arterial pressure was low, the resulting fall in blood pressure was often irreversible. "When the systemic arterial pressure had fallen to shock levels for more than one minute, it could usually be restored only by pressor drugs," the investigators report.

They also found that "The lung stretch systemic arterial pressure relation was abolished after cutting the vagi in the neck (4 experiments) or after blocking them by cold (3 experiments). The phenomenon reappeared, however, when the cold-blocked vagi were permitted to rewarm."

These and other observations from the studies were interpreted by the Los Angeles workers as evidence for the occurrence of reflex systemic blood vessel dilation on stretch stimulation in the lungs.

"The clinical implication of these observations is readily apparent for conditions in which the functional pulmonary circulation is temporarily excluded, such as during cardio-pulmonary bypass," the workers state. "The depression of the systemic arterial pressure to shock levels for indefinite periods as observed occasionally in our experiments correlates with the clinical observations of Beecher et al that an increase of positive pressure in the airways may be dangerous in patients in poor circulatory conditions. Since the reflex is elicited not only by insufflation, but also by mechanical stretching, the need for gentleness in the surgical manipulation of thoracic organs is re-emphasized."

PHYSIOLOGICAL EXPERIMENTS AFFORD UNIFIED CONCEPT OF CONTROL OF CARDIAC OUTPUT

Experiments conducted in the Heart Institute Laboratory of Cardiovascular Physiology indicate that the sympathetic

nerves to the heart play a major role in regulating cardiac output through a direct effect on the heart muscle fibers. The findings indicate that these cardiac sympathetic nerves govern the force of the heart's contraction, and thus cardiac output. These respond to arterial blood pressure stimuli received in pressure sensitive areas, such as the carotid sinus. A unified concept of the basic control of cardiac output has resulted from these findings.

Studies conducted by Dr. Stanley J. Sarnoff and his co-workers in the NHI's Laboratory of Cardiovascular Physiology, concerned with the integration of the performance characteristics of the heart as a pump with other cardiovascular mechanisms and control elements, have resulted in a unified concept of the basic control of cardiac output by the central nervous system in precise accordance with the blood flow and energy requirements of the organism. It has been shown that the curve which describes the fundamental relationship between initial filling pressure or myocardial fiber length and the external ventricular work produced (Starling's Law) can be variably and reproducibly shifted over wide ranges to the right or left by grading the impulse frequency applied to the cardiac nerve emanating from the isolated left stellate ganglion. It was demonstrated that such stimulation could effect up to 1000 percent increases in the left ventricular work produced at any given filling pressure.

It was then shown that these nerve pathways to the heart respond to changes in baroreceptor stimulation (blood pressure in the carotid sinus). Such blood pressure produced changes in the left ventricular work (at the same filling pressure) which were five to ten times as great as the changes in peripheral vascular resistance or heart rate resulted. The latter have previously been thought to be the dominant regulatory influences of baroreceptor stimulation.

The implications of these observations together with other available information makes it appropriate to account for the fundamental control of cardiac output on the following basis.

The tissues of the organism change their respective local resistances to flow through blood vessels in accord with local energy requirements. Increased energy requirement produces a local dilation of blood vessels and thus decreased resistance to flow. As a result arterial pressure tends to decline. The baroreceptor stimulation that results from the falling blood pressure not only increases heart rate, as previously shown, but also produces an increase in heart muscle contractility so that, from the same filling pressure, a vastly increased cardiac work can be produced, thus maintaining the head of arterial pressure, from which the various tissues can "accept" more or less flow in accordance with their activity and thus local vascular resistances.

These findings were presented at the Federation Meetings.

SURGERY

DIRECT COOLING OF BLOOD AND
MEMBRANE OXYGENATOR AS AIDS
TO SIMPLER CARDIAC SURGERY

A team of Tennessee surgeons reports a method of open heart surgery which places a minimum demand for equipment and methodology on the surgeon and yet provides him with prolonged direct vision access to the interior of the heart. This is accomplished by an unusual combination of hypothermia with a low flow heart-lung apparatus. Direct cooling of the blood (rather than the usual immersion hypothermia of the entire patient) by routing it through a simple heat-exchange coil reduces the oxygen needs of the patient enough to permit further simplification and safety in employment of a Teflon membrane pump-lung more nearly approaching the normal lung than the commonly used oxygenators. The new method, perfected in dogs, is reported ready for clinical application.

Drs. E. Converse Peirce, III, C. Harwell Dabbs, William K. Rogers, and Freeman L. Rawson of the East Tennessee Tuberculosis Hospital and Acuff Clinic, Knoxville, describe the method in the Journal of the Tennessee State Medical Association. The method was developed in National Heart Institute grant-supported work.

"Although much good open heart surgery has been done using equipment or methods that are inherently dangerous," the surgeons state, "it is now time that we re-examine our approach to this important field and eliminate all equipment and procedures that depend primarily on good luck or the manual dexterity of the operating surgeon."

To permit proper intracardiac procedures to be carried out with maximum safety, prolonged direct vision access to the entire inside of the heart is needed while adequate oxygenated blood is provided the brain and other vital organs. This can be accomplished by pump-lungs that maintain the patient's entire circulation, they acknowledge, but unfortunately few medical centers have such elaborate equipment. The great reduction in oxygen requirement brought about by hypothermia has also led to the adoption of immersion cooling to extend the time of open heart visibility. But, as the workers point out, cooling the entire patient in an ice bath is also complicated and inefficient (chilling and rewarming apparatus is relatively unwieldy and the vital organs are adequately cooled only after skin and muscles are markedly refrigerated).

"By directly cooling the blood, organs are cooled in proportion to their blood flow," the authors explain in advocating their method. "Organs that have a high blood flow and, therefore, a

large need for oxygen, receive a much larger volume of cold blood. Consequently the temperature falls faster in them and a greater saving in oxygen-need is brought about for a given number of calories than is possible with general cooling of the body..."

Further simplification of the problem caused by direct vision intra-cardiac surgery can be met by using a Teflon membrane pump-lung, which more nearly approaches the normal lung than those commonly employed, the surgeons report. The strong, thin Teflon membranes readily admit the diffusion of oxygen into the contained blood. The absence of bubbles when blood and aerating atmosphere are separated by membranes eliminates the requirement for filters and antifoaming agents.

Details of the technique as employed with the membrane lung and simple stainless steel heat-exchange coil are given in the publication. The method is described as used in the experimental laboratory, but the authors write that it is now sufficiently advanced to recommend clinically.

STRAIN GAUGE USED ON HUMAN HEART DURING SURGERY TO MEASURE CONTRACTILE FORCE

The principle of the resistance wire gauge, used in industry for stress analysis, has been adapted by NHI grantees at the Medical College of

South Carolina for measuring the force of contraction of the human heart muscle. At NHI it has now been applied safely in 47 surgery patients in the first measurements of the direct effects of digitalis and "adrenalin" compounds on human heart muscle contractility. The findings appear clinically applicable and encourage further use of the method in human subjects.

The measurements, which have already prompted some potentially lifesaving new guidelines for the preparation of heart surgery candidates, were reported by Drs. Robert D. Bloodwell, Leon I. Goldberg, Eugene Braunwald, Joseph W. Gilbert, John Ross, Jr., and Andrew G. Morrow of the Clinic of Surgery at the annual meeting of the American College of Surgeons in Atlantic City.

Previously the responses of the human heart muscle to drugs could only be inferred from clinical studies of blood pressures and flow, and from more direct measurements in animals. The NHI studies now show the strain gauge arch to be a safe and practical method for assessing, in man, the direct effects of drugs and anesthetics on myocardial contractility, and to provide a means of evaluating various procedures applied during cardiac surgery.

The Walton-Brodie strain gauge arch used in these studies resembles industrial strain gauges in that it is basically an

element of "resistance" wire, which responds to stretching tensions with variations in its resistance to electrical current. In the Walton-Brodie instrument, the tiny coiled thread of resistance wire is housed in an inch-long metal cylinder to insulate it against body fluids. By harmlessly anchoring the two ends of the device to the myocardial fibers, the muscle contractions are made to stretch the resistance wire. With the passage of a weak electrical current through the wire, the heart muscle's contractile force is thus translated into an electrical expression, which can be made to deflect the line traced by a recording device.

Applied earlier by engineers to the measurement of mechanical quantities in industry, such as acceleration, pressure, and impact force, the device was recently adapted to the heart by R. P. Walton, O. J. Brodie and others at the Medical College of South Carolina. These workers, and another team headed by T. D. Darby from the same institution who later gave it its first application in humans, were supported in their studies by Heart Institute research grants.

Applying the Walton-Brodie strain gauge arch in 47 patients undergoing heart surgery, the NHI team has learned that digitalis compounds greatly increase the contractile force of the non-failing, as well as the failing heart muscle. The widely held view that the tonic effect of digitalis on the heart muscle is limited to patients with congestive heart failure has inhibited preoperative use of this valuable drug in heart surgery candidates who might have derived some protection from it. In other drug studies, it was found that the clinically important "adrenalin" compounds, epinephrine and norepinephrine, had comparable effects in increasing the contractile force of the heart muscle.

The recently adopted "anoxic" method of elective cardiac arrest, by which the heart is temporarily stopped by intermittently clamping off its entire outflow through the aorta, was evaluated in twelve of the patients at open heart operation. Although the measured contractile force of their heart muscle disappeared quickly on clamping the aorta, it also returned quickly to control levels after the clamps were removed and blood once again moved through the coronaries. The prolonged myocardial depression that has been reported to occur following drug-induced elective cardiac arrest did not occur with the anoxic method. This suggests the anoxic method be employed wherever elective cardiac arrest is necessary, Dr. Bloodwell recommended to the American College of Surgeons.

Extension of the studies using the strain gauge arch to the evaluation of other clinically important drugs and procedures is anticipated.

TRANSPLANTED DOG HEARTS
FUNCTION SATISFACTORILY
FOR SHORT PERIODS

The technically successful transplantation of hearts in dogs, with survival for periods up to seven and one-half hours, has been

reported by Drs. Watts R. Webb, Hector S. Howard, and William A. Neely of the University of Mississippi. The investigators, presenting their results in the Journal of Thoracic Surgery, state that of twelve such transplanted hearts, satisfactory cardiac function with normal blood pressure was restored in ten.

Three major problems confront investigators working in the field of heart transplantation. These are the needs for 1) practical surgical techniques for removing and re-implanting the heart, 2) methods for keeping both the heart and the recipient alive in the interim, and 3) a way of overcoming the immunological or "rejection" response of the body to tissues from another individual. Results of the NHI grant-supported dog studies by the Mississippi workers indicate that considerable progress is being made toward solution of the first two of these.

In their transplantation procedure, the Mississippi researchers carefully removed the hearts from matched pairs of dogs, taking care to leave vessel "stubs" of adequate length. Hearts to be transplanted were either perfused with a special saline solution to flush all blood from the heart, or injected with the anti-coagulant, heparin, to prevent blood clotting. The hearts were then stored for 2 to 4 hours at about 39 degrees Fahrenheit in a special nutrient solution. The circulation of the recipient animals was maintained by a heart-lung machine until donor hearts were installed, warmed, and capable of carrying the full circulatory load.

Metal coupling devices for the stitchless joining of severed vessels were used for making all venous connections. Aorta and pulmonary artery connections were generally sutured. When the coupling devices were also used for the aorta and pulmonary artery connections, heart insertion could be completed in 22 minutes instead of the usual 40 minutes.

During early development of their techniques, the investigators were plagued by "countless mistakes and failures." Of twelve technically successful transplants, however, ten were able to maintain normal blood pressures from 30 minutes to 7½ hours, displaying essentially normal heart action as revealed by electrocardiograms. The researchers believe air embolism was responsible for the two failures. Blood loss by oozing from extensive raw surfaces was found to be the greatest hazard of the procedure.

In commenting on the possibility of future heart transplantation in humans, the investigators concluded that "Obviously, many refinements and further developments are necessary in many phases of this complex procedure before such will be possible. In addition, the unsolved problem of the immunologic responses will prevent permanent survival of any transplanted homologous organ at this time."

NHI ADOPTS BETTER METHOD FOR DETECTING RESIDUAL HEPARIN AFTER SURGERY

The Heart Institute Clinic of Surgery has adopted plasma thrombin time (the time it takes for the patient's plasma to clot after thrombin has been added) as the single most sensitive and practical method for judging the adequacy of heparin neutralization after this anticoagulant has been used in patients requiring the heart-lung machine.

The heparin administered to patients undergoing surgery with the heart-lung machine must be neutralized at the conclusion of the operation if excessive bleeding is to be avoided. Injections of protamine are used for this purpose because of the uniquely safe, fast, and specific heparin-neutralizing action of this protein.

Measurement of the amount of protamine required to enable a sample of the heparinized patient's blood to clot (the "protamine titration" method) has been advocated as the method of choice for determining the dose of protamine necessary to restore normal blood clotting. However, Dr. James C. Peden of the Clinical Center Clinical Pathology Laboratory and Drs. James W. McFarland and Andrew G. Morrow of the NHI Clinic of Surgery, report that protamine titration is an unnecessarily cumbersome method, and often fails to indicate the presence of significant amounts of circulating heparin. These workers sought a better method in measurement of the time it takes for a sample of the patient's plasma to clot after addition of the clot-promoting enzyme, thrombin.

Twenty-five patients subjected to cardiopulmonary bypass were studied in comparing the two methods. In eight patients protamine titration was used to determine the dose of protamine necessary to neutralize their heparin. In seven of the eight, the dose indicated was far less than that ultimately required to return their clotting time to normal.

Even when blood clotting time had actually returned to normal, residual anticoagulant could be demonstrated by prolonged plasma thrombin times in 18 of the 25 patients studied.

A paper describing these findings has been published in Blood, the Journal of Hematology.

**GASEOUS OXYGEN PERFUSIONS
USED TO MAINTAIN THE BEAT
OF BLOODLESS DOG HEARTS**

The beat of dog hearts deprived of their blood supply can be maintained for periods of $2\frac{1}{2}$ to 8 hours by perfusing the coronary circula-

tion with gaseous oxygen combined with CO_2 , Drs. David C. Saviston, Jr., James L. Talbert, Lee H. Riley, Jr., and Alfred Blalock of the Johns Hopkins University Hospital and School of Medicine report in the Annals of Surgery.

The NHI grant-aided investigators replaced the coronary blood flow of 22 dog hearts with a humidified oxygen (95 percent) and carbon dioxide (5 percent) gas mixture. Ten hearts were isolated for study in a transparent plexiglas chamber maintained at a constant temperature and humidity and twelve were perfused in place within the animals' chests. Effects of the gas perfusions on visible contractions, contractile force, and electrical activity of the hearts were recorded.

Fourteen additional hearts were isolated for control experiments in which they were either perfused with compressed air, 100 percent nitrogen, other gases, or not perfused. All hearts, whether isolated or in situ, were initially perfused with blood to institute strong, normal contractions, and then flushed of all blood with an oxygenated saline solution.

Duration of the visible beat in the ten isolated hearts perfused with gaseous oxygen, as seen through the plexiglas chamber, ranged from $2\frac{1}{2}$ to 8 hours and averaged 5 hours and 7 minutes. In some instances, electrocardiographic activity continued after visible contraction had ceased and in one instance was observed four hours after the last visible beat. The hearts' contractile force, measured directly by a strain gauge arch sutured to the ventricular surface, "... characteristically remained quite forceful for the first two to three hours and then became gradually weaker," the researchers report.

In the control experiments, three hearts perfused with compressed air were maintained for more than four hours. In three hearts perfused with 100 percent nitrogen, rapid weakening and complete cessation of the heart beat occurred within 4, 15, and 28 minutes respectively. Five hearts that received no gas perfusions following administration of oxygenated saline, failed in 8 to 28 minutes.

The investigators perfused the 12 in situ hearts with gaseous

oxygen for 25-30 minutes. Then, following flushing with saline, they attempted to reestablish normal blood flow. They report that normal circulation was restored in nine of the twelve animals for one to 48 hours, notwithstanding the inadequate blood flow to the brain and lack of flow to the rest of the body which had occurred during the oxygen perfusion period. They state: "The studies are not considered to accurately reflect the true results of long term survival due to the prolonged anoxia of other organs which occurred during the perfusion."

The perfusion studies show that not only may gaseous oxygen be utilized to maintain the heart's vitality for prolonged periods, but that the subsequent restoration of normal circulation is also possible. The findings may have important applications in maintaining cardiac metabolism during open-heart surgery.

HEART INSTITUTE ABANDONS POTASSIUM FOR ARRESTING HEARTBEAT DURING SURGERY

Studies by the Clinic of Surgery indicate use of potassium citrate to deliberately quiet the heart entails more danger of irreversible

heart failure than does the anoxic method, which prevents interfering heart motion by intermittently clamping off the outflow of blood through the aorta.

The adoption by cardiac surgeons in recent years of coronary potassium infusions for deliberately stopping the heart marks an important development in the history of intracardiac surgery. But with the increasing use of potassium for such "elective" cardiac arrest, heart surgeons have become increasingly concerned about the difficulty of restoring a strong and effective heart-beat after prolonged operations using elective arrest. Some surgeons have come to question whether the heart should be stopped at all for many intracardiac operations. Others question the chemical methods, and in some quarters interest is focusing on an "anoxic" method of arrest that interrupts blood flow through the heart by intermittently clamping off the aorta.

The Heart Institute Clinic of Surgery has completed animal studies comparing the three major methods of elective cardiac arrest--the original potassium method, the recently adopted "anoxic" method of clamping the aorta, and acetylcholine, another chemical that temporarily stops the heartbeat. Both chemicals caused a severe depression of ventricular function when the period of arrest exceeded ten minutes, but the function of this muscle was virtually unchanged after heart motion was restricted by intermittently clamping off the outflow through the aorta (anoxic arrest) for as long as thirty minutes.

"Although flaccid paralysis of the heart certainly facilitates

the repair of many intracardiac anomalies, the hazard of the method is so great as to outweigh this advantage," the NHI group concluded. "A dry and quiet field can be secured by intermittent aortic occlusion alone, and this technique is applicable in virtually all procedures in which aortotomy is not necessary. When the aorta must be open for long periods, as in the repair of aortic lesions, direct coronary perfusion has been found the technique of choice."

Participating in the studies under the direction of Dr. Andrew G. Morrow, Chief of the Clinic, are Drs. James A. McFarland, John A. Waldhausen, Louis B. Thomas, Nina S. Braunwald, Joseph W. Gilbert, Robert D. Bloodwell, and William P. Cornell. The studies were reported at meetings of the American Heart Association and the American College of Surgeons.

**CORONARY CIRCULATION
IMPROVED BY NEW
SHUNT OPERATION**

A new operation for combating coronary artery disease has been reported in which a fraction of the heart's output of venous

blood is made to bypass the lungs and mix with arterial blood in the left atrium.

Developers of the operation, Drs. Stacey B. Day and C. Walton Lillehei of Minneapolis, report in the journal Surgery that joining the pulmonary artery and left atrium side-to-side and establishing an opening, or shunt, between them increases within one month's time the amount of blood available to the heart muscle by stimulating the formation of new blood vessels. The newly-formed blood vessels connect major branches of the coronary arteries.

In their studies, which were partly supported by a National Heart Institute training grant in cardiac surgery, the surgeons found that the shunt operation protected dog hearts during experimental closure of the circumflex coronary, a major heart artery. Subsequent clinical application of the operation in a 47-year-old man abolished his anginal pain, eliminated the need for about 100 nitroglycerin tablets weekly and enabled him to resume moderately heavy physical work.

The new operation is unlike other heart revascularization procedures in that it stimulates heart blood vessel formation by reducing the amount of oxygen in the heart's own blood supply. Previous workers had already demonstrated that reduced oxygen tension greatly increases coronary blood flow by dilating blood vessels. Drs. Day and Lillehei reasoned that such an increase

in blood flow would also provoke new artery formation. Their operation diverts oxygen-poor venous blood from the pulmonary artery to the left atrium where it mixes with freshly-oxygenated blood, thus lowering the oxygen tension of the blood pumped to the heart muscle. The scientists calculate that only about 20 percent of the unoxygenated blood pumped by the heart is detoured by the shunt--the remaining 80 percent continues its normal course through the pulmonary artery and the lungs, thence to the left atrium as fresh, oxygenated blood.

The Minnesota surgeons report a striking difference in survival rates between shunt dogs and normal control dogs when subjected to experimental coronary occlusion. Pulmonary artery-left atrial shunts, established two to four weeks earlier, were closed prior to tying-off the circumflex coronary artery. Of the ten experimental shunt dogs, 90 percent, or nine animals, survived coronary ligation one hour or longer. Only seven, or 28 percent, of the 25 normal control dogs survived a comparable period.

That the increased survival rate was due to an increase in arterial connections between the coronary branches was demonstrated by preparing heart injection-corrosion casts. Exact replicas of the heart's circulatory system are made by this technique, in which the blood vessels are injected with a liquid plastic and all heart tissue removed with acid to free the hardened plastic cast.

The investigators are of the opinion that shunts may be left permanently open. They have, however, developed two simple methods for closure that do not necessitate shunt re-exposure, for use in the event lowered oxygen tension is not well tolerated or adverse pressure differences result from the shunt procedure.

RECORDING OF SOUNDS INSIDE	Intracardiac phonocardiography, the
HEART AIDS DIAGNOSIS OF	recording of sounds within the
INBORN HEART DISEASE	heart, provides a means of deter-
	mining the location of heart sounds

and murmurs to a degree not heretofore obtainable, NHI grantees have found.

Dr. David H. Lewis and his co-workers at Philadelphia General Hospital and the U. S. Naval Air Development Center at Johnsville, Pennsylvania, report in the journal Pediatrics on their adaptation of a Navy underwater microphone to pick up sounds and murmurs within the heart that characterize inborn heart disease. The technique of recording sounds within the heart, called intracardiac phonocardiography, was carried out in 63 patients with congenital heart disease and 11 patients in the pediatric age range who were

found after study not to have heart disease.

The basis of their work rests on the application to medicine of acoustic techniques developed by the U. S. Navy for undersea warfare. One of several Navy underwater microphones, the elemental tubular hydrophone, utilizes an activated barium titanate crystal as the sound receiving element. Its adaptation to intracardiac phonocardiography was accomplished by making the sound receiving element small enough to be incorporated into a specially designed cardiac catheter and by designing suitable amplifiers to allow the signal from the catheter to be fed into the usual instruments on which phonocardiograms are recorded.

The sound catheter, which was developed with NHI grant aid, and its use in making intracardiac phonocardiograms of the normal heart, were reported in 1957 by Dr. Lewis and his co-workers. The 1959 report in Pediatrics describes its use in mapping intracardiac sounds that characterize congenital heart malformations and presents findings demonstrating its clinical value in the diagnosis of these heart defects. Phonocardiographic tracings are reproduced which represent the sounds heard within the heart and blood vessels of the conditions known as patent ductus arteriosus, pulmonic stenosis, ventricular septal defect, aortic stenosis, combined pulmonic stenosis and ventricular septal defect, atrial septal defect, and coarctation of the aorta.

The investigators conclude from their studies that intracardiac phonocardiography has an important place in the diagnosis of congenital heart disease because of its ability to sharply localize heart sounds and murmurs. This degree of localization apparently occurs due to the hitherto unsuspected quality of acoustic damping provided by the blood.

"At our present level of information, intracardiac phonocardiography does not replace any existing techniques," the workers state. "However, as more information is gathered it might well do this." In most of the cases reported, use of the sound catheterization technique gave additional information confirming diagnoses previously made. But on occasion it enabled positive diagnosis when blood samples, intravascular pressures, and clinical picture were equivocal. There were also instances where the intracardiac sounds served to rule out a lesion which was suspected to exist on clinical grounds.

"For these reasons," they summarize, "we feel that intracardiac phonocardiography is a very valuable aid to the diagnosis of congenital heart disease and that it should be made a part of the routine studies done at the time of cardiac catheterization."

NEW KNOWLEDGE AND METHODS

STRUCTURALLY DAMAGED ENZYME CAN SPONTANEOUSLY REBUILD, RECOVERING LOST ACTIVITY

NHI biochemists have been taking apart the molecular structure of an enzyme--a "representative" protein--to learn more about how the structures of proteins determine their various functions in living matter. Like other proteins, the enzyme molecule under study (ribonuclease) consists of a long chain of repeating links (peptides) coiled and folded into a characteristic three-dimensional shape.

The NHI workers have found that the shape of the molecule is influenced by the existence of four "cross-links", or bonds between the sulfur atoms of certain links in the chain. Breaking these four "disulfide" cross-links was found to strip the enzyme of its power to break down ribonucleic acid--its normal function in the cell.

This deformed and inactive molecule has now been found capable, under suitable conditions, of spontaneously rebuilding all four of the ruptured cross-links, with full recovery of its lost power as an enzyme.

These findings are regarded as adding significantly to the body of knowledge which is expected, ultimately, to enable biochemists to read in the structure of proteins the nature of their role in living matter.

Drs. Christian B. Anfinsen, and Frederick H. White, who made these findings in the NHI Laboratory of Cellular Physiology and Metabolism, reported them at the Federation meetings.

POLYVINYLPYRROLIDONE USED AS SUBSTITUTE PROTEIN IN NEW DIAGNOSTIC TEST

In the NHI Laboratory of Cellular Physiology and Metabolism, studies of the metabolism of plasma proteins have led to the development of a clinically useful test for abnormal leakage of macromolecules from the circulation through the digestive tract.

Dr. Robert S. Gordon of the Metabolism Section developed the test to facilitate studies of plasma protein transport and metabolism. For such studies, very small amounts of a biochemically inert macromolecule, which could be isotopically labeled, and then injected into the circulation to simulate the large natural molecules of circulating protein in their passage through the cell membrane barriers, were needed.

Polyvinylpyrrolidone (PVP) possessed most of the desired attributes. Developed by the Germans during the '30s, PVP was one of the advances in polymer chemistry that attended the development of modern plastics technology. Widely used as a blood substitute by the Germans during World War II, PVP has been injected into hundreds of thousands of persons without known ill effect. It does not react to body chemicals and is partly excreted unchanged by the kidneys in normal persons.

Since existing methods for preparing PVP with a radioactive label (Iodine¹³¹) didn't provide a preparation satisfactory for accurate quantitative studies, Dr. Gordon developed a method of preparing labelled PVP. This can be done conveniently and cheaply with I¹³¹ and remains stable through vigorous chemical treatment. This work was published by Dr. Gordon in the Journal of Polymer Chemistry as "The Preparation of Radioactive PVP for Medical Use."

The test was applied in the study of "hypercatabolic" hypoproteinemia, a rare and puzzling type of plasma protein deficiency. In the more common hypoproteinemias, loss of serum protein is usually through some comparatively obvious route like the skin, as in burns, or the kidney, as in nephrosis or the production of protein may be reduced, as in malnutrition or liver disease. In the "hypercatabolic" form, however, abnormally rapid catabolism, or metabolic destruction, of the protein has been hypothesized since no other route of its disappearance could be found. Dr. Thomas Waldmann and other workers in the National Cancer Institute participated in these clinical studies, which are published in Annals of Internal Medicine.

The NCI and NHI investigators applied the test in control subjects without hypoproteinemia, and in subjects with hypoproteinemia due to kidney disease. They found little or none of the radioactivity appearing in the feces of control subjects, in contrast to its appearance in large amounts in the feces of all of nine "hypercatabolics" studied, coincident with disappearance of radioactivity from the plasma.

Dr. Gordon, in a preliminary publication in The Lancet, suggests that the term "hypercatabolic" be reserved for hypoproteinemic cases whose disease can be shown to be caused by accelerated catabolism. He suggests "exudative enteropathy" as a more appropriate term for those cases, demonstrated by the PVP test, in which hypoproteinemia is due to abnormal intestinal permeability to large molecules.

Two pharmaceutical concerns, Abbott and Squibb, are investigating the possibility of production of I¹³¹-labeled PVP on a commercial scale.

PORTABLE RADIO SYSTEM FOR TRANSMITTING PHYSIOLOGICAL DATA OVER SHORT DISTANCES

A completely transistorized radio transmitting and receiving system weighing less than two pounds, which transmits physiological

information (such as electrocardiograms) for distances of about 200 feet indoors and 500 feet outdoors, is reported by a physician and an engineer working under an NHI grant at the University of Nebraska.

Dr. F. Lowell Dunn and Harold G. Beenken of Omaha have already applied a model of their new radio telemetering system to electrocardiogram reception from patients transmitting from other parts of a building or exercising on a treadmill. Among the electrocardiographic tracings thus obtained, some of which were published in their report in the Journal of the American Medical Association, is one showing the sudden onset of a disturbance of heartbeat during exercise. The radio-transmitted information made it possible to terminate the exercise before the disturbance of heartbeat became more serious.

Use of more powerful transistors will greatly increase the range, and models far smaller than the two-pound one now in use can be made by using miniature and subminiature components.

The investigators report that the system can be adapted to counting, temperature, pressure curves, and electrical and mechanical sensing. They have discussed its application in many projects, such as the monitoring of rectal temperature and/or electrocardiogram in a tractor operator working in a field, electroencephalographic monitoring in a hospital ward or conference, recording muscular activity during training exercises, pressure measurements from various parts of the foot during walking, and electrographic recording and monitoring from operating and recovery rooms without connecting wires.

"Modern medical practice often utilizes formidable masses of instrumentation both in the ward and operating room," Dunn and Beenken point out. "The stability and ease of operation of this transmitter offers the possibility of eliminating a few of the wires draped around the subject and at the same time notably reduces the explosion hazards due to the very low voltages used and the completeness of shielding possible. This isolation of the transmitter makes it much less susceptible to stray fields, artefacts, and noise."

BIOCHEMISTS FIND ENZYMATIC MECHANISM FOR SYNTHESIZING OF NOREPINEPHRINE IN BRAIN

The brain has been shown by Heart Institute biochemists to make its own norepinephrine, a well known peripheral nerve

impulse transmitter, or neurohormone, recently found to occur in regions of the brain regulating organic functions that are beyond voluntary control. Dr. Sidney Udenfriend and Cyrus Creveling of the Laboratory of Clinical Biochemistry found that dog brain contains the enzyme, dopamine-beta-oxidase, that makes norepinephrine from its precursor, dopamine. Measuring isotopically the activity of the enzyme in different brain tissues, these investigators have begun to map the regions of the brain in which the pace of norepinephrine manufacture is most intense. By thus delineating the brain structures wherein norepinephrine plays its neurochemical role, such research may help determine the exact function of this hormone in the central nervous system.

The recent discovery of norepinephrine in the brain and the emerging pattern of its distribution there suggests it might be involved centrally, as it is elsewhere in the body, in the biochemistry of nerve centers that regulate autonomic functions.

Norepinephrine is known to be derived from the dietary amino acid, tyrosine, and its immediate precursor is generally acknowledged to be dopamine. Conversion of dopamine to norepinephrine by the enzyme dopamine-beta-oxidase has been observed many times in body tissues other than brain.

Dr. Sidney Udenfriend and Cyrus Creveling of the NHI Laboratory of Clinical Biochemistry have demonstrated, for the first time, the presence of dopamine-beta-oxidase in brain, measuring the activity of this enzyme in the conversion of dopamine to norepinephrine in various brain tissues. This is published in Federation Proceedings.

Their studies show that the enzyme is highly localized in certain areas, with large amounts present in the hypothalamus and caudate nucleus, little in the cerebellum, and no measurable amount in the cerebral cortex.

The rate of norepinephrine synthesis by this enzyme was found to be as intense in some areas of the brain as it is in the medulla of the adrenal glands, where the concentration of hormone is more than a thousand times as great. This finding suggests that the measure of norepinephrine alone in such tissues does not necessarily reflect the rate of its utilization there.

"More detailed measurements of dopamine-beta-oxidase activity in the central nervous system along with the other catalysts

and intermediates should make it possible to map out in detail those areas of the brain in which norepinephrine plays its physiological role," the investigators explain. "Such information may eventually prove useful in determining the exact function of the catecholamines in the central nervous system and to determine which biosynthetic steps may be rate-limiting with respect to norepinephrine formation and release."

KRYPTON FOUND SUPERIOR FOR DETECTION OF ABNORMAL VEIN CHANNELS IN LIVER DISEASE

Abnormal channels of blood flow, or shunts, often develop spontaneously between the special (portal) system of veins of the liver, and the main (systemic) venous system. This happens when the scarring damage (cirrhosis) of chronic liver disease obstructs the flow of portal blood through the liver from the intestine. The pressure of the blocked portal blood backs up into the digestive tract as far as the veins of the esophagus, often bulging them (esophageal varicose veins) and even rupturing them (esophageal hemorrhages). Bleeding from such esophageal varices often demands the creation of artificial portal-systemic shunts to relieve the pressure in the portal system. These surgical procedures save many lives, but they are risky, especially for debilitated alcoholics, who are especially prone to cirrhosis. So the demonstration of existing shunts is often important both in evaluating the patient for surgery, and in evaluating the results of surgery already done.

But the detection of surgically and naturally acquired shunts has been impossible without complicated X-ray techniques requiring the introduction of contrast substances into the abdominal circulation.

Drs. Robert T. L. Long, Carlos R. Lombardo, Eugene Braunwald, and Andrew G. Morrow of NHI have developed a superior method for demonstrating such shunts using a radioactive isotope of krypton gas (Kr^{85}). This method was reported in Clinical Research Proceedings.

Cardio-green, an indicator dye used in diagnostic studies of congenital intracardiac shunts, was included with the Kr^{85} in the NHI studies. Dissolved in saline, both indicators were injected simultaneously into the portal or intestinal venous circulation of nine dogs which had been prepared with temporary portal-systemic shunts. Dilution of the Kr^{85} , which passes out of solution immediately on contact with air, was measured by Geiger counter in the dogs' exhaled air. Dilution of the dye was measured photoelectrically in blood drawn from a leg artery or from the left atrium.

Both methods were found effective. When the shunts were clamped off the indicator substances were delayed measurably (in crossing the liver) before passing to the systemic circulation and the detecting devices.

The data showed the Kr^{85} method to be most sensitive. Closing off the temporary shunts delayed the arrival of the isotope in the lungs an average of 30.1 seconds, while the arrival of the dye in the arterial blood was delayed only 14 seconds. An advantage of the Kr^{85} method is that blood sampling is not required.

PENICILLIN INJECTIONS MOST
EFFECTIVE RHEUMATIC
FEVER PREVENTIVE

Three-year results of a continuing study of rheumatic fever prophylactic methods show the superiority of intramuscularly

injected, long-acting penicillin over oral penicillin or sulfadiazine in preventing streptococcal infections and rheumatic fever recurrences. Earlier data from the study, published in 1957, had been difficult to evaluate as it was not known how faithfully patients adhered to their prescribed treatment schedules.

The study, which is partially supported by a National Heart Institute grant, is being conducted at Irvington House, Irvington-on-Hudson, New York, by a team of investigators from Irvington House and the New York University College of Medicine, New York City. These investigators are: Drs. Alan R. Feinstein, Harrison F. Wood, Jeanne A. Epstein, and Angelo Taranta; Rita Simpson, statistician, and Esther Tursky, head nurse. Seven other workers collaborated with the principal investigators in the New York study. Their results appear in the New England Journal of Medicine. In the study, 391 children and adolescents known to have had previous attacks of rheumatic fever were divided into three groups. Each group was given one of three drugs: sulfadiazine, 1 gram daily by mouth, 200,000 units daily of buffered penicillin potassium G by mouth, and 1,200,000 units of benzathine penicillin G injected intramuscularly every four weeks. At monthly intervals during the three-year study, the patients underwent clinical, bacteriologic and serologic examinations to detect streptococcal infections and rheumatic activity. Special techniques, including patient-interviews and the counting of unused pills, were instituted during the third year to determine how faithfully patients took their oral medications.

"The data show unequivocally that (penicillin) injections are more effective than either sulfadiazine or penicillin by mouth, in the dosage schedule used, for the prevention of streptococcal infections and rheumatic fever," the investigators report.

An additional finding of the study was that, in the dosage schedules used, oral sulfadiazine was as effective and possibly superior to oral penicillin in preventing streptococcal and rheumatic attacks. The scientists regard this as rather surprising because sulfadiazine is a bacteriostatic agent, not a bactericidal agent.

The current studies will be expanded so that the apparent superiority of sulfadiazine can be tested against a double daily dose of penicillin.

PARTIAL VITAMIN C ACTIVITY OF D-ASCORBIC ACID AFFORDS CLUES TO ROLE OF VITAMIN C

A new experimental approach for studying the physiological role of natural vitamin C (L-ascorbic acid) is provided by findings at

NIH of partial vitamin C activity in the synthetic analog, D-ascorbic acid. Although D-ascorbic acid has been reported to lack the vitamin activity, NHI and NIDR workers found it can cure some, but not all, of the symptoms of scurvy in guinea pigs when given in proper dosage. The nature of the partial vitamin activity of the D analog indicates a dual role for the natural vitamin in the body.

The findings were made by Drs. J. J. Burns and Peter G. Dayton, Laboratory of Clinical Pharmacology, National Heart Institute and Dr. Harold Fullmer, National Institute of Dental Research. The findings were presented by Dr. Burns at the Fourth International Congress of Biochemistry in Vienna, Austria.

Initial studies by these workers showed that D-ascorbic acid is excreted much more rapidly by vitamin-C-deficient guinea pigs than is L-ascorbic acid. This suggested a possible explanation for its reported inability to cure scurvy. In order to test this point Dr. Burns and his associates administered D-ascorbic acid to vitamin-C-deficient guinea pigs in such a way as to achieve concentrations in the body similar to those required for L-ascorbic acid.

Under these conditions D-ascorbic acid could cure certain symptoms of scurvy. For instance, even in small doses the structural analog was able to maintain the weight and survival of severely scorbutic guinea pigs. In addition, D-ascorbic acid was able to correct the defective dentine produced in scurvy much in the same way as L-ascorbic acid. However, despite the normal appearance of the guinea pigs treated with D-ascorbic acid, they were found upon autopsy to have severe joint hemorrhages characteristic of the disease.

Thus, the results show that D-ascorbic acid is able to replace L-ascorbic acid for some of the activities of

vitamin C. This is of importance since it indicates a dual role for vitamin C: one that is specific which requires only L-ascorbic acid, and the other that is non-specific in which D-ascorbic acid and perhaps other compounds with the same oxidation-reduction potential can substitute. These observations have furnished a new experimental approach for studying the vitamin's role in the body.

Dr. Burns has long been interested in both the chemistry of ascorbic acid and the physiologic problem of its requirement as a vitamin by primates and guinea pigs--a unique requirement which has profoundly influenced the spread of civilization over the earth. Characteristically, the free exploration of this problem across disciplinary and institutional boundaries at NIH is providing a variety of information of value to the different disciplines and institutions concerned. In the NHI Laboratory of Chemical Pharmacology, Drs. Burns and Dayton are using pharmacologic agents (such as vitamin C substitutes) as research tools to gain fundamental physiologic knowledge. Joining forces with Dr. Fuller in the NIDR Laboratory of Histology and Pathology not only adds the skills of a pathologist to those of chemist and pharmacologist (Burns and Dayton) for the NHI study, but also provides Dr. Fuller and the dental sciences with new information on the pathology of scurvy, a disease of great dental importance.

**NERVE STIMULI INFLUENCING
HEART CONTRACTIONS ALSO
INFLUENCE URINE FLOW**

During experiments designed to effect hypertension by electrical stimulation of the sympathetic nerves to the heart,

investigators in the NHI Laboratory of Cardiovascular Physiology observed that such stimulation produced not only an increase in the contractility of heart muscle and hypertension but also a significant increase in the volume of fluid excreted by the kidneys. J. P. Gilmore of NHI announced this finding at the meeting of the Federation of American Societies for Experimental Biology.

The possibility that the increased urine flow, or diuresis, might be a direct result of the hypertension was ruled out in experiments in which the vagus nerves were sectioned in the neck. This procedure significantly diminished the diuresis even though blood pressure rose still higher. Sectioning of the vagus nerves below the heart, however, does not modify the diuresis, suggesting that the receptor components of the reflex are, in whole or part, contained within the thoracic cage.

The characteristics of the diuresis (rapidity of response, increased secretion of chloride and electrolytes, and increased glomerular filtration rate) are such as to suggest that it is not mediated via changes in the circulating levels of aldosterone and/or the antidiuretic hormone.

To date, the results of the experiments being performed in this laboratory suggest that the increased urine flow which accompanies cardiac sympathetic nerve stimulation results from reflex stimulation of pressure receptors located in whole or part in the chest and that the vagus nerves are part of the afferent pathway of the reflex. The receptors appear to be stimulated via the dynamic changes in arterial and/or ventricular pressure which occur when the cardiac sympathetic nerves are stimulated. Thus, pressure receptors not only modify myocardial contractility and peripheral resistance but would also appear to play a role in the regulation of body fluid volume.

FEAR MOBILIZES BODY FAT AS
EMERGENCY FUEL FOR LIFE
PROCESSES

An NIH study shows that fear increases the free, or unesterified, fatty acids (UFA) in the blood.

Earlier studies conducted at NHI over the past few years indicate that the fatty acids stored in adipose tissue are made available for life processes by release into the blood in an "unesterified" state (not bound, as esters, to cholesterol and other lipids in the lipoproteins). Studies published during 1957 by Dr. Robert S. Gordon, Jr., of NHI indicate that these unesterified fatty acids (UFA) increase in the blood during fasting, reaching levels sufficient to provide energy for the metabolic activities of virtually all body tissues except the brain.

More recently Dr. Philippe V. Cardon of NIMH teamed with Dr. Gordon in a study of the effects of fear on the UFA in the blood of 15 normal volunteers. The "fear" to which the young volunteers were subjected was the unexpected (and unfulfilled) threat of a painful injection.

In most of the subjects this hoax produced a rapid rise in the plasma UFA to peak levels within 10 minutes. In some the UFA more than doubled (two subjects) or tripled (one subject).

The findings from this study are published by Drs. Cardon and Gordon in the Journal of Psychosomatic Research.

"On the basis of the data presented we believe that psychic phenomena can change plasma levels quickly and profoundly," they write. "It is reasonable to infer that in a large majority of individuals plasma UFA increases when fear is experienced."

"Confronted by an emergency," the investigators suggest, "an individual burns fat wherever possible and conserves glucose for use by the central nervous system, expecting, as it were, not to eat until the emergency passes."

GAS CHROMATOGRAPHY EXTENDED
TO ANALYSES OF GASES
IN BIOLOGICAL FLUIDS

Dr. Lloyd Ramsey at Vanderbilt University in Nashville has reported a way to apply gas chromatography in the analysis of the

gases in blood plasma and other biological fluids. He uses a vacuum to extract the gases from the sample without heat before subjecting them to analysis. By first extracting the gases from the fluid, the problem of the intolerance of existing equipment for wet samples is surmounted. By using a vacuum method to do the extracting, the destructive effects of heat on the sample are avoided.

The pioneering advances of the English scientists, James and Martin, in gas chromatography have focused worldwide scientific attention in recent years on this remarkably sensitive method for separating closely related chemicals in vapor form.

But the method has not been applicable in the analysis of the permanent gases in body tissues and fluids because the heat customarily used to volatilize the gases in liquid samples coagulated and oxidized other constituents of biological samples, altering their gas concentrations and introducing intolerable errors. Also, the adsorption column of the apparatus, which separates the constituents of the sample, will not work if it is wet.

With vacuum and agitation, Dr. Ramsey is able to coax the gases from one milliliter samples of biological fluids prior to their chromatographic analysis. All permanent gases excepting argon (which present column materials cannot separate from oxygen) can be separated and analyzed by any of several column materials, according to Dr. Ramsey's report. Dr. Ramsey is especially enthusiastic about the usefulness of the method for oxygen analyses, for which he has subjected it to particularly stringent tests.

"The remarkable sensitivity of the instrumentation to minute changes in oxygen content in relatively small samples of biological fluids, the excellent stability of the instrumentation, and the accuracy of the method offer opportunity for its widespread use in biological research and clinical medicine," he writes in Science. "The method makes possible accurate analysis, on small samples, of the concentration of biologically important gases present, or changing in time, in biological solutions. It seems likely that many determinations previously made by means of the Van Slyke, Haldane, Scholander, and Warburg methods can now be made with equal or better accuracy and simplicity and in less time. The oxygen tension of arterial blood can easily be calculated from the oxygen content of plasma as determined in this manner. Such determinations previously required exceptional

technical skill and even then were less accurate and more time consuming than determinations made according to the method described."

Dr. Ramsey's work is supported by an NHI research grant.

CANCER-CAUSING HYDROCARBONS TRANSPORTED BY LIPOPROTEINS NHI STUDIES INDICATE

Dr. Joel Avigan of the NHI Metabolism Section recently devised a method for incorporating cholesterol into human lipoproteins

in vitro. An immediately useful consequence of this advance is the availability of better cholesterol preparations for intravenous injection in studies of lipid metabolism. (The lipoprotein -- a "natural" vehicle for cholesterol transport -- is superior to the detergent-stabilized cholesterol suspensions previously used when this insoluble lipid had to be injected into the circulation).

More recently Dr. Avigan found that the water insoluble cancer-producing hydrocarbons can, like cholesterol, also be dissolved in human or animal serum, where they are similarly incorporated into the lipoproteins. This suggests that serum lipoproteins might represent the vehicle by which such cancer-producing agents are ordinarily transported throughout the body.

In the journal Cancer Research, where he reports his in vitro method for incorporating the carcinogenic hydrocarbons into lipoproteins, Dr. Avigan also suggests uses for the method in cancer research. "The transport mechanism of carcinogens and the relative affinities of the tissue cells for the various compounds could be conveniently studied with the aid of the soluble preparations...", he writes.

Among the cancer-producing hydrocarbons studied were a number of anthracenes, related to the carcinogens in coal tar, as well as fluorene derivatives, including 2-acetylaminofluorene.

Since 1915, when application of coal tar to the ears of rabbits led to the first reported instances of experimental cancer, hundreds of industrially produced and naturally occurring hydrocarbons and their derivatives have been tested and a large proportion have been found capable of causing cancer in experimental animals. Such cancer-producing hydrocarbons are found in many substances obtained from coal tar and petroleum, in smoke, soot, auto exhaust, paving materials and in the atmosphere.

Like cholesterol and most of the body's other normally occurring fatty substances, these cancer-producing hydrocarbons do not generally dissolve in water -- yet, when orally administered,

they may be absorbed into the aqueous medium of the blood and distributed throughout the body.

Past attempts to follow orally administered hydrocarbons in the circulation did not reveal how they were made soluble in the blood. Incorporation into the lipoproteins appears to be the answer, Dr. Avigan's findings suggest.

ELECTRICAL POTENTIAL SEEN
AS FACTOR IN INTESTINAL
ABSORPTION-SECRETION

Workers in the NHI Laboratory of
Kidney and Electrolyte Metabolism
have accurately measured the dif-
ference in electrical potential

across the wall of the large intestine and correlated this with water and salt movement in an effort to gain information concerning the important but largely unknown mechanism by which this organ absorbs and secretes electrolytes and water.

Circulating balanced electrolyte solutions through loops of large intestine in anesthetized dogs, Drs. Irving Cooperstein and Stanley Brockman used an extremely sensitive potentiometer to measure the minute differences in voltage between contacts placed against the inner and outer surfaces of the large intestine. Water movement was indicated by changes in concentration of a phenol red dye, and changes in the concentration of salts were measured chemically and with radioactive isotopes.

The findings showed that when the intestine is in an absorbing state, the surface in contact with the intestinal contents is electrically negative to the blood. So the movement into the blood of the sodium, an electrically positive ion (cation), is "uphill", or against the electrical gradient. This implies the existence of an energy-requiring transport mechanism. The chloride, an electrically negative ion (anion), responds to the electrical gradient and moves into the blood passively, or in a "downhill" direction. The water follows the salt.

When the large intestine is in a secreting state, bicarbonate is actively transported from the blood into the intestinal lumen, taking water with it.

A finding from these studies which may throw additional light on the nature of the transport mechanism involved is that the digitalis compound, strophanthidin, known to inhibit cation transport in other tissues, reduces the electrical potential across the intestine, interfering with the movement of sodium and bicarbonate.

Drs. Cooperstein and Brockman have reported the results of their studies in the Journal of Clinical Investigation and in Clinical Research.

SPECIAL DRUG-INACTIVATING
MECHANISMS ABSENT IN
NEWBORN MAMMALS

The biochemical mechanisms
(enzyme systems located in liver
microsomes) that normally termin-
ate drug action in the body are

absent in newborn mammals during the first week of life, National Heart Institute scientists have found. The investigators are Drs. Bernard B. Brodie, W. Robert Jondorf, and Roger P. Maickel of NHI's Laboratory of Chemical Pharmacology.

The investigators compared the ability of mice and guinea pigs of various ages to metabolize several commonly used drugs. In the studies, guinea pig livers were homogenized and incubated with various test drugs. The drugs used included analgesics (aminopyrine and phenacetin), a barbiturate (hexobarbital), a laxative (phenolphthalein), and monomethyl-4-aminoantipyrine. The in vitro experiments showed that the drug-destroying enzyme systems are absent in fetal and newborn guinea pigs, but appear during the first week of life and require about eight weeks to develop fully.

Studies in vivo confirmed the liver incubation studies by demonstrating that newborn mice are unable to metabolize aminopyrine, phenacetin, or hexobarbital. An additional potential danger of giving drugs to the newborn was observed in studies of the duration of hexobarbital action. The investigators found that responses to this barbiturate varied greatly between different age groups of mice. Very small doses of the drug (10 mg./kg.) increased the sleeping-time to 360 minutes for one-day-old mice and to 107 minutes for 7-day-old mice, in contrast to adult mice who were barely affected by the drug and had a sleeping-time of less than five minutes.

These results suggest that a lack of liver enzyme systems is responsible for the extreme sensitivity of newborn animals to the barbiturate and perhaps to other drugs as well.

Stressing the clinical implications of the findings, the investigators state: "That newborn mammals are unable to metabolize these compounds is of obvious importance in considering the use of drugs in childbirth and for newborn infants."

The findings were presented at the Federation Meetings in Atlantic City and published in the journal Biochemical Pharmacology.

CHLOROTHIAZIDE PROMISING
IN TREATMENT OF TOXEMIA
AND EDEMA OF PREGNANCY

The powerful diuretic and hypo-
tensive drug, chlorothiazide,
produces good results and minimal
side effects when used to relieve

the edema of toxemia and of normal pregnancy, according to the clinical data of NHI grantees at the University of California

Medical School. Studied in 50 women with toxemia and 100 with the edema of normal pregnancy, oral and intravenous chlorothiazide was seen to produce striking increases in urinary sodium chloride and water excretion, with corresponding loss of body weight in most of the patients. All tolerated the drug without side effects.

The unusual diuretic effects of chlorothiazide, first reported in 1957 by investigators at Merck and Co., have been studied by various workers under experimental conditions, in the edema of congestive heart failure, and in hypertension, where a significant fall in blood pressure accompanies the accelerated sodium, chloride, and potassium excretion caused by this drug.

Dr. Nicholas S. Assali and coworkers Lewis L. Judd and Norman Mondz, of the University of California Medical School, reported in the Journal of the American Medical Association a clinical and metabolic study of the chlorothiazide diuresis in the edema of normal pregnancy and of toxemia of pregnancy.

Detailed metabolic studies were carried out on 15 patients hospitalized with toxemia and treated with oral and intravenous chlorothiazide. The effects of the drug on water and electrolyte metabolism and kidney function were observed in these patients and blood and urinary sodium, potassium, chloride, carbon dioxide, and pH, as well as weight, blood pressure, and the balance of electrolyte intake and output were recorded in this connection.

In addition to these metabolic studies, clinical observations were reported on 135 outpatients, 100 of whom were normal except for pitting leg edema. The other 35 showed not only edema, but also varying degrees of the hypertension and urinary protein loss which characterize the toxemia of pregnancy.

Both oral and intravenous chlorothiazide were reported to produce a striking increase in sodium, chloride, and water excretion, with corresponding relief of the edema in most of the patients with both conditions (only 15 of the 135 did not show significant weight loss the first week). Potassium excretion was also increased by the drug, but less dramatically.

The workers found the action of the drug to be prompt, reaching its peak on the first day. Intravenously, the maximum effect was noted within 30 minutes.

When treatment was stopped, salt and water were again retained for a time, apparently as a sort of rebound from the salt depletion of the drug, but this reaction was not found to be

of sufficient magnitude to seriously compromise the good diuretic effect.

No consistent effect of chlorothiazide on blood pressure was seen in this study, an observation which seems at odds with reports on the use of this drug, in hypertension. A possible explanation, offered by the Los Angeles workers, is that they were administering chlorothiazide alone instead of combining it with other drugs as is customary for hypertension. Also, their periods of observation may have been too short to include long term changes in blood pressure.

Satisfactory diuretic effects with minimum side effects can be achieved in edema and toxemia of pregnancy with daily oral dosage anywhere within the 500 to 2,000 milligram range, according to the findings of this study.

NHI WORKERS DEMONSTRATE ALDOSTERONE-STIMULATING AGENT IN CARDIAC EDEMA

NHI and NIAMD investigators have found through experiments in dogs that a substance capable of stimulating secretion of the electrolyte-regulating hormone, aldosterone, from the adrenal glands appears in the circulation in cardiac edema. In their continuing efforts to determine its exact nature, origin, and the stimulus which elicits it, the workers are proceeding on the assumption that the substance is a hormone and that its secretion is involved in the sequence of events leading to cardiac edema.

Release of the aldosterone-stimulating substance in edematous circulatory disease was shown through cross-circulation experiments in dogs by Dr. James O. Davis and Nicholas A. Yankopoulos of NHI, and Drs. Ralph E. Peterson and Bernard Kliman of NIAMD. This work is published in the Journal of Clinical Investigation.

Dr. Davis' group feels that release of the aldosterone-stimulating substance could be a main link in the sequence of events leading to cardiac edema. Each new link found in this sequence presents a new point for possible clinical attack on the widespread and costly health problem of cardiac edema.

Although isolated only five years ago, aldosterone is accepted by most authorities as the primary adrenal hormone for regulating renal salt and water excretion. This has led to intensive efforts by many groups to find the mechanism that governs aldosterone secretion--especially its oversecretion in heart, kidney, and liver diseases.

The basic plan of the NHI study consisted of cross-circulation of blood from donor animals with edema-producing circulatory

disorders (constrictions of the inferior vena cava) into the adrenal glands of normal recipients. In seven pairs of animals, blood was circulated from chronically edematous donors which were secreting six-fold the normal amount of aldosterone into the isolated recipient adrenals. In every instance, aldosterone secretion by the recipient glands increased in response to the blood from abnormal donors (the average increase was 129%). In eight pairs, blood was cross-circulated from normal donors into isolated recipient glands to provide control data on the effects of cross-circulation alone. This effect was not significant.

Aldosterone secretion by donors and recipients was measured isotopically in adrenal vein blood. The levels of aldosterone in the general circulation are too low to be measurable by this method, so the high levels found in adrenal vein blood in these studies represent secreted, not donated, hormone.

PUMP DESIGNED TO RELIEVE
FAILING HEARTS REACHES
STAGE OF CLINICAL TEST

Use of the surgical pump-
oxygenator to temporarily
relieve the workload of human
hearts damaged by heart attacks

was first reported in the literature of 1957. Now a simple pump, lacking an oxygenator and designed especially for the long-term support of failing hearts, has been announced from NHI grant-supported work at Tufts University School of Medicine and Boston City Hospital.

The new apparatus, which collects blood from a vein and pumps it into an artery, was developed by James F. Dickson, Neal A. J. Hamer, and James W. Dow, physicians now with the Presbyterian Hospital, Philadelphia. The pump has been brought through experimental stages in dogs and has reached the level of clinical testing, although its usefulness in man has not yet been demonstrated.

The first "veno-arterial pumping" in a patient with this apparatus is described in the A.M.A. Archives of Surgery. The pumping was continued for 26 hours in an unsuccessful effort to sustain the life of a 52-year-old man suffering from intractable heart failure.

"Though the patient's condition was not improved, harmful effects from the apparatus were not apparent during the procedure or at post-mortem examination," the physicians report. "Except for platelet depression, no serious damage to the formed elements of the blood or the coagulation mechanisms was found."

"The feasibility of prolonged veno-arterial pumping in man has been demonstrated."

The apparatus is described as consisting essentially of a transparent flexible plastic (Silastic) collecting chamber in a rigid Plexiglas case, mounted in parallel with two pumps, also Plexiglas-encased Silastic, which are driven by graded impulses of compressed air.

Venous blood was drained from the patient into the tiny (200 ml.) collecting chamber through a canula in the jugular vein and pumped at a rate of 700-1000 ml. per minute, into a leg (femoral) artery.

In the earlier experiments with the apparatus, veno-arterial pumping was maintained as long as 53 hours in dogs without serious effects, Dr. Dickson's team reported. In the single disappointing clinical trial the pump itself performed according to expectations and no detriment resulted from its use, but the patient showed no benefit from its use either. Its designers speculate that a greater flow from the pump might be more effective. Assuming that further experience might show veno-arterial pumping to be of benefit in heart failure, the investigators emphasize a number of advantages in the design of their apparatus.

The closed system prevents all contact with air and the danger of artery-clogging bubbles and clots that such contact might promote. The small size and simplicity make for economy, mobility, and easy servicing, and preclude dangerous changes in the patient's blood volume.

Improvements in pump design and operative technique expected to increase the scope of the veno-arterial pumping are under study by the investigators.

HYDROXYPROLINE METABOLISM STUDIED AS APPROACH TO COLLAGEN DISEASE

By combining and improving existing techniques for measuring hydroxyproline, NHI workers were able for the first time to measure accurately urinary hydroxyproline in men. They found that eight of ten patients with Marfan's syndrome (a collagen disease known for its effects on bone, eye, and heart development) studied at NHI had higher levels of urinary hydroxyproline than the average found in twelve normal control subjects.

The study of hydroxyproline metabolism offers a direct approach to the unknown etiology and pathology of collagen disease because this amino acid occurs in high concentration only in the connective tissue protein, collagen. Collagen binds together and supports skin, tendon, bone, cartilage, the lining of arteries, and some of the inner structures of the heart, as well as various

other body tissues and structures. Any of these may be affected by collagen disease.

The abnormality of hydroxyproline metabolism--presumably the first reported in a collagen disease--and the improved methods for measuring urinary hydroxyproline are reported in The Journal of Laboratory and Clinical Medicine. The authors of this paper are Drs. Chozo Mitoma, Thomas E. Smith, Frances M. DaCosta and Sidney Udenfriend of the Laboratory of Clinical Biochemistry and John D. Davidson and Albert Sjoerdsma of the Laboratory of General Medicine and Experimental Therapeutics.

CATHETERIZE HEART'S LEFT
CHAMBERS VIA NEEDLE FROM
RIGHT, ACROSS SEPTUM

NHI surgeons are now placing catheters in the hard-to-reach left chambers of the heart from the more accessible right heart

by penetrating the vertical partition, or septum, separating the left and right chambers (atria). The "transseptal" method possesses unique advantages over other ways of catheterizing the left heart, and is an extremely valuable new aid to the safe and accurate diagnosis of cardiovascular disease.

With the growing awareness among both surgeons and physiologists that techniques for learning about heart functions have been lagging behind methods of repairing hearts, the NHI Clinic of Surgery has been concentrating on the development of better methods for the measurement of heart functions, both to provide clinical medicine (surgery and cardiology) with badly needed ways of mapping diseases to be treated, and to provide medical research with better tools for investigation.

One of the most important advances to result from this effort during 1959 has been the development of the transseptal method of inserting catheters into the left heart. The method was developed and tested in 37 dogs by Surgeon John Ross, Jr., and has been applied by him, with Cardiologist Eugene Braunwald and Surgeon Andrew Morrow, in more than 100 patients in the Heart Institute's Clinic of Surgery.

All available methods for threading these information-sensing devices into the left heart have been previously used in the Heart Institute and found to have limitations. The most widely used method of puncturing the skin to aim the catheter-bearing needle at the left heart across the chest cavity was largely abandoned at NHI in recent years in favor of a safer and surer approach down the windpipe. However, this "transbronchial approach" is uncomfortable to the patient, whose conscious co-operation is required, and thus is often impossible in children.

The "transseptal" method takes the easy downstream route into the right atrium via a leg vein. Thus right heart catheterization is readily carried out in the conventional way, a standard catheter being advanced through the leg vein into the right ventricle and pulmonary artery for obtaining information concerning right heart function. To cross the septum into the left heart, the standard catheter is replaced with a shorter catheter and a special needle is then inserted within it. The needle is advanced, with fluoroscopic guidance, until it is appropriately positioned (still sheathed in the catheter) against the atrial septum. Then the needle is advanced just enough beyond the catheter tip to puncture this partition. Pressure data and indicator dilution curves can then be obtained from the left atrium through the needle itself, and a second small catheter can be advanced through this needle into the ventricle and sometimes further into the aorta.

Because the method involves no external heart puncture, it avoids the risks of hemorrhage from the heart, damage to the lung, and air-leakage into the chest cavity that may attend other methods. No significant complications have resulted from its use in any of the 100 patients.

The method is simple enough to be performed by individuals competent in the usual catheterization techniques. It permits recording of data from both right and left chambers in a single study. It involves no appreciable discomfort, so it can be used in conscious patients, even children, to obtain data on heart function in a basal, or relaxed, state. (Pressures have been measured continuously for several hours in the left ventricle of individual patients.)

A report on the clinical use of the transseptal method has been published in the American Journal of Cardiology. An exhibit explaining the method, prepared for use at medical and scientific meetings, had its first showing at the 1959 Scientific Sessions of the American Heart Association in Philadelphia.

MYOSIN "SUBUNITS" OBTAINED
BY DIGESTION ARE IRREGULAR
FRAGMENTS OF THE MOLECULE

Myosin, the major contractile protein of muscle, can be split by proteolytic enzymes into large fragments. These fragments

have been called "subunits" by some authors and various speculations have been presented on the possible arrangement of these subunits in the molecule.

The work of Dr. Elemer Mihalyi and Dr. William F. Harrington in the Heart Institute Laboratory of Cellular Physiology and

Metabolism has now proven that the "subunits" are actually not pre-existing structural units but digestive fragments of the molecule.

Study of the fragmentation reaction may provide valuable information on the structural characteristics of fibrous proteins such as the collagen of connective tissue and the fibrinogen of blood clotting, as well as the contractile protein of muscle.

Muscular contraction is brought about by some yet unknown configurational change of the myosin molecule and the energy required by this process is liberated also by the myosin molecule, through its enzymatic function directed toward the breaking of a high energy phosphate bond. Thus, the myosin molecule seems to be a complete unit to convert chemical energy into mechanical work. Knowing its complete structure is, therefore, a prerequisite to the understanding of muscular contraction.

Myosin is a very large molecule, much too large to be attacked by structural investigations in its intact form. It was thought that the proteolytic fragmentation of the myosin, since it yields well-defined fragments, may be used advantageously in structural investigations, especially to establish connections between the different functions and certain localized areas of the molecule. On the other hand, the proteolytic process itself may shed some light on the structure of the molecule.

The fragmentation of the myosin molecule was followed in the ultracentrifuge and at the same time the number of peptide bonds split also was determined by physicochemical methods and also confirmed by chemical analysis. It appears that some of the peptide bonds are split at a rate about ten times faster than that of the remainder. This fast reaction accounts for the formation of the large fragments. Some optical evidence suggests that parts of the myosin molecule are formed of a tightly coiled chain, whereas others are much looser and randomly folded. Apparently the enzyme attacks with much greater ease the random portions and thus liberates the tightly coiled fragments, giving the erroneous impression of liberation of preexistent subunits.

This fragmentation reaction may prove of more general use. There are indications that collagen, the major protein of connective tissue, and fibrinogen, a protein responsible for blood clotting, are degraded by proteolytic enzymes in much the same way. Thus, at least with fibrous proteins, it is possible that the proteolytic breakdown of the molecules, under suitable conditions, can be used to estimate the proportion of the molecule in the tightly coiled form as opposed to the random portion. So far, only optical methods have been used for this purpose.

The NHI workers reported the findings on myosin fragmentation in Biochimica et Biophysica Acta.

PROGRAM HIGHLIGHTS

HISTORIC 'REPORT TO NATION'
PRESENTED TEN YEARS' GAINS
AGAINST HEART DISEASE

At the request of Senator Lister Hill and Representative John E. Fogarty, the National Heart Institute and the American Heart

Association sponsored a Report to the Nation on ten years of progress against the cardiovascular diseases. The Report was presented by a panel of six of the country's most prominent cardiologists and medical scientists at the U. S. Department of Commerce Auditorium on February 19.

In addition to the approximately 1000 people who attended the Report, newspapers, press associations, magazines, reprints, radio networks, TV networks, and a motion picture carried the Report to the American people in the ensuing weeks.

The distinguished panel of speakers highlighted those heart research advances made possible through the cooperative efforts of the voluntary agency, the American Heart Association, and its Federal partner, the National Heart Institute. Both agencies have now completed ten years of service in combating the cardiovascular diseases. Through their joint efforts, research has been greatly expanded, and effective programs of public education, professional education, and community service have been established.

This coordinated attack, the speakers emphasized, has speeded the development of new advances in diagnosis, treatment, prevention and rehabilitation, with the result that much suffering has been alleviated, lives saved or prolonged, and individuals with cardiovascular disease enabled to continue as productive citizens.

The six panelists were: Dr. Howard B. Sprague, Lecturer on Medicine at Harvard Medical School, who spoke on diagnosis; Dr. Paul Dudley White, President of the International Society of Cardiology Foundation, who spoke on prevention; Dr. Irvine H. Page, Director of Research at Cleveland Clinic, who spoke on treatment with special reference to atherosclerosis; Dr. Robert W. Wilkins, Director of Medicine at Boston School of Medicine, who spoke on treatment with special reference to hypertension; Dr. Michael E. DeBakey, Professor of Surgery at Baylor University, who spoke on treatment with special reference to surgery; and Dr. Robert W. Berliner, Associate Director in Charge of Research at the National Heart Institute, who spoke on basic research.

In addition to the panel presentation, there were brief remarks by the Hon. Lister Hill, U. S. Senator, Alabama; the Hon. John E. Fogarty, U. S. Representative, Rhode Island; Leroy E. Burney, M. D., Surgeon General, U. S. Public Health Service; Francis L. Chamberlain, M. D., President, American Heart Association; and James Watt, M. D., Director, National Heart Institute.

**EPIDEMIOLOGY PROBLEMS AIRED
HEART METHODOLOGY EXAMINED,
MEETING SUGGESTS SOLUTIONS**

The problems of methodology common to epidemiological studies of cardiovascular diseases were the topic of a three-day con-

ference held in April at Princeton, New Jersey. The National Heart Institute and the American Heart Association were co-sponsors of this conference. Representing NHI among the eighty participants were Dr. James N. Hundley, Chairman of the Committee on Diet, Physical Activity and Biochemical Measurements; Dean E. Krueger, Executive Secretary for the conference; and Drs. Luther L. Terry, Robert P. Grant, and Joseph A. Bragdon.

Epidemiology has been called the main discipline of preventive medicine. Drawing upon statistical methods, all phases of medicine, and the natural sciences, it charts the occurrence and distribution of various diseases in population groups and attempts to isolate the factor or factors responsible. Often these factors can be effectively isolated by selecting populations for study which differ widely in the incidence or severity of a certain disease but which are very similar in all other respects except for the factors (such as diet, degree of physical activity, etc.) which might account for this difference.

In recent years epidemiologists have exploited many of these experiments of opportunity and have gained valuable insights into possible causes of cardiovascular diseases. However, one of the major problems encountered by epidemiologists is the lack of standardized methods for collecting, classifying and evaluating epidemiological data. For this reason data collected in one study frequently cannot be compared with that collected by other studies in the same epidemiological areas; thus an invaluable means of enriching the findings of these studies is lost. This problem must be solved if the full potential of epidemiology as a research tool is to be realized.

In attacking this problem, the Conference sought to develop workable criteria and standardized procedures for collecting, classifying, and evaluating epidemiological data; to resolve

areas of doubt or disagreement; and to point out problem areas requiring further study.

Prior to the session at Princeton, the four committees, comprising nine pre-conference groups, had met to consider problems in their particular areas. Preliminary reports of these meetings were prepared for study by the participating scientists.

Among the problems considered at the conference were the following: the design and analysis of epidemiological studies; criteria, standardized procedures and controls for biochemical measurements; means of assessing physical activity and body form for population groups and for individuals within groups; means for collecting and analyzing dietary data; standardized criteria and methods for blood pressure measurement, electrocardiography, and morphology; and methods for determining and evaluating cultural, societal, familial, psychological, and genetic influences on cardiovascular disease.

The proceedings of this conference will be published by the American Heart Association in early 1960.

NEW STREP TEST VALIDATED:
RAPID DIAGNOSIS POSSIBLE,
H.D.C.P. TRIALS PROVE

On October 29 Secretary Flemming announced at a news conference that the fluorescent antibody technique for

the rapid diagnosis of streptococcal infections--some of which are precursors of rheumatic fever and rheumatic heart disease--had been validated in field tests. The tests, which were conducted by the Communicable Disease Center and financed by the Heart Disease Control Program, were run in cooperation with State and local health departments and pediatric hospitals in Tennessee, North Dakota, Colorado, Illinois and Maryland. Throat swabs taken from over 1,200 persons were tested by the standard method, which takes several days, and by the fluorescent antibody technique, which takes 2 to 3 hours. Results showed the fast test to be as accurate as the slower method.

The new technique, developed by the Communicable Disease Center Laboratory in Atlanta, utilizes a fluorescent dye in combination with the specific antibodies for these streptococci. When placed in contact with these streptococci, the fluorescent antibodies attach themselves to the organisms, causing them to glow under ultraviolet light and making positive identification possible. The test can be made with as few as 200 organisms, and the disease identified, while conventional methods often require as many as 100 million organisms to give good results.

The Heart Disease Control Program, the Communicable Disease Center and the National Heart Institute are helping to extend the use of the test as rapidly as possible through the following actions:

1. Local personnel have been trained and equipment left on indefinite loan in the areas that participated in the field tests.
2. About 40 Public Health Service physicians assigned to State and local health departments held a special meeting in Philadelphia on October 27 to determine the best ways in which they could help health departments, medical societies, and heart associations throughout the country to take advantage of the new research avenues which the fast strep test had opened.
3. A 2-week training course for laboratory personnel of 12 State health departments will be held at the Communicable Disease Center in January, 1960. Materials and equipment will be loaned to PHS laboratories as soon as they have personnel trained to use it.
4. Financial assistance is provided through grants from the National Heart Institute for research projects and through State grants-in-aid for purchase of equipment.

TWO PRIMATE CENTERS PLANNED,
LOCATIONS TO BE SELECTED,
TWO MILLION EARMARKED

On October 27, Dr. Willard H. Eyestone joined the National Heart Institute staff to take charge of the Primate and Veterinary Grants Program. He is a member of the National Advisory Committee on Primates, which functions as a study section to evaluate applications for research grants involving use and care of primates. This committee has reviewed a number of applications contemplating establishment of primate research centers, and will make recommendations to the National Advisory Heart Council regarding scientific merit and priority consideration. Two such centers are planned, at university locations to be decided upon in 1960. Two million dollars have been earmarked for the NHI program.

Because of their physiological similarity to human beings, non-human primates such as monkeys, baboons, and chimpanzees have become increasingly important in scientific research. Much of the progress made in recent years against polio, mental disease, and tuberculosis would have been impossible without these primates; conversely, lack of them has hindered

research in the fields of cardiovascular disease, gerontology, and hematology. The planned centers will operate as independent research units, and in addition their facilities will be available to visiting scientists from foreign countries and to scientists from American universities and industrial laboratories.

<p>NEW AIDES FOR EPIDEMIOLOGY, ANTHROPOLOGISTS ENLISTED, MAY FIND DISEASE CLUES</p>	<p>Anthropologists specially trained to collect epidemio- logical data in the course of their regular studies may uncover valuable leads that can be exploited by epidemiologists in such fields as cardiovascular disease, diabetes, and cancer. This new approach is presently under study by the Geographic Disease Study Section, established last year under the joint sponsorship of NHI and NIAMD and headed by Dr. James Hundley.</p>
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The Center has awarded a contract for the preparation of a guidebook for anthropologists, setting forth criteria and methods for collecting useful epidemiological data and specimens. These will include blood and urine samples; blood pressure readings; physical measurements such as height, weight, body build and degree of fatness; quantitative measurements of individual food intake; and overt symptoms of disease. These samples, measurements, and other applicable data will be sent to NIH for medical and statistical analysis. When the findings prove promising, more exhaustive studies of the same population group will be undertaken by specialized epidemiological teams.

A study that will provide an effective test of both the method itself and of the techniques being evolved is now being considered. This study will be carried out among the Herrero, an isolated African group which has subsisted for generations on a diet derived chiefly from dairy products.

<p>BLOOD CONFERENCE SPONSORED, CLOTTING FACTORS STUDIED, NOMENCLATURE REVIEWED</p>	<p>Supported in part by an NHI grant, the Fourth Meeting of the International Committee on Blood Clotting Factors was held August 23-26 in Montreaux, Switzerland. More than eighty scientists from sixteen countries attended. Representing NHI at the meeting was Dr. Eleanor M. K. Darby, Head of the Conference and Reports Section of the Grants and Training Branch.</p>
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The Committee was established to devise an international system of nomenclature for blood clotting factors, based on (1) clarification of the properties and functions to which multiple and overlapping names had been assigned in the past and (2) the development of criteria for the formal recognition of any old or new factor as a definite entity. The achievement of this goal is needed primarily to make possible more effective communication among experimental scientists. This need has been heightened by the related need of physicians to understand and manipulate the coagulation mechanisms in treating patients with actual or threatened coronary, cerebral, or peripheral thromboses. Anticoagulants have been widely used in efforts to prevent complications following myocardial infarction and cerebral vascular occlusions. However, this has been done in spite of the very controversial status of knowledge concerning the mechanism of operation of both anticoagulants and blood clotting factors, and the lack of a satisfactory basis for the administration and evaluation of such therapy. As long as the scientists are unable to agree on mechanisms or nomenclature, the practicing physician may not be able to fully apply the knowledge of anticoagulant therapy.

The first two days of the meeting were devoted to a program on new clotting factors which featured papers and discussions on the Stuart-Prower Factor, Factor X, and the plasma thromboplastin antecedent (PTA). On the following day came the business meeting of the Committee itself, which was devoted to problems of nomenclature and the reports and recommendations of its working sub-committees. The Stuart-Prower Factor, the Hageman Factor, Factor X, and PTA were considered for inclusion with the eight factors already formally recognized and designated by the Roman Numeral System adopted at the September 1958 meeting. The Stuart-Prower Factor was accepted and designated as Number X in the series; "Factor X" was withdrawn; and the other two were recommended for further study by technical sub-committees.

It was agreed that the next conference to be sponsored by the Committee would be held at Princeton, New Jersey in September of 1960, and would be concerned with the fast-developing field of fibrinolysis research. Fibrinolysis is a process which involves the body's own mechanisms to prevent clots or to lyse clots already formed. Emphasis will be on the basic aspects of various fibrinolytic and thrombolytic processes which have been under investigation both in this country (with substantial grant support from NHI) and abroad.

MEETING RECOMMENDS ACTION,
FIVE DIET STUDIES RESULT,
SEEK DATA, METHODS

The major problems encountered in collecting and evaluating dietary data in epidemiological studies of cardiovascular disease

were studied exhaustively by the Sub-committee on Methodology of Diet Appraisal at the Conference on Methodology held at Princeton in April. Growing out of specific recommendations made by this group, a number of studies designed to aid in the solution of major problems in this area have been launched by the Geographic Disease Study Section. The intent is to provide data, methods, or other information which will increase the effectiveness, accuracy and comparability of dietary studies being done on a wide scale in this country on the epidemiology, pathogenesis, etiology, and evolution of cardiovascular diseases. Among these studies:

--Extension and revision of standard food composition tables is being carried out in cooperation with USDA and other agencies. One problem area is the large number of widely used, commercially prepared mixes and ready-to-eat foods of varied composition being placed upon the market each year. So great has been the influx that the testing of these new products for inclusion in food tables has fallen far behind. Another problem area is the more accurate evaluation of mixed dishes (fried potatoes, beans cooked with pork, etc.), recipe dishes (stews, etc.) and regional dishes. For these food tables increasingly greater attention is being given to the saturated and unsaturated fatty acid content of common foods.

--The use of electronic computation for faster, more efficient processing of dietary data is under study. An IBM-650 has been in use for the past year with the Framingham Study and has proven to be of great value. This trial run will be useful in reprogramming and updating the computer using the more reliable and more detailed specific food values (such as those for fatty acids) now becoming available. It is planned to provide computer processing as a service to grantees engaged in field studies in cardiovascular disease, first on a research basis, later as a routine service.

--A collection, compilation, and critical evaluation of published and unpublished work on methodology for diet appraisal is being undertaken under the direction of Drs. James Hundley and Marjorie Whiting. In this connection Mrs. Rose Ernsberger has been assigned to the Geographical Disease Study Center to assist in the translation and compilation of recent Russian literature

in the nutrition field. At present there is virtually no information available on either the extent of this literature or its quality.

--A contract has been awarded to the University of Michigan for the development of new methods to determine dietary intake. The emphasis in this study will be on simplified procedures for large scale surveys which will provide data of sufficient accuracy to provide a basis for more intensive follow-up studies. The study will combine the efforts of nutritionists, sociologists, epidemiologists, and statisticians.

--Through a cooperative arrangement with FAO and USDA, a biochemist was assigned to FAO headquarters in Rome to assess the available data on food consumption, with special attention to the amount and type of fat consumed, of selected national populations throughout the world. Following an evaluation of material now available, attention will be directed to the design of studies in selected countries which will produce data pertinent to current research in cardiovascular disease.

LIPID JOURNAL LAUNCHED,
PUBLISHED QUARTERLY,
AIDED BY NHI GRANT

The Journal of Lipid Research,
a new quarterly devoted to the
promotion of basic research in
the lipid field, has begun

publication with an issue dated October 1959. Financial support is derived entirely from a Heart Institute grant and from subscriptions received from individuals and institutions.

Published by Lipid Research, Inc., a non-profit group, the journal will contain original articles dealing with the chemistry, biochemistry, enzymology, histochemistry, and physiology of the lipids.

The new publication's editorial staff is headed by Dr. D. B. Zilversmit, of the University of Tennessee, with Dr. J. H. Bragdon, NHI, and Dr. Jules Hirsch, of the Rockefeller Institute, as Associate Editors. Dr. Daniel Steinberg, also of the Heart Institute, is a member of the editorial board.

International in scope, the Journal lists prominent scientists from Canada, Sweden, France, Germany, England, Belgium, Israel and Japan as corresponding editors. Drs. Edward H. Ahrens, Jr., Herbert E. Carter, Irvine H. Page and W. H. Sebrell, Jr., comprise the Advisory Board.

HIGHLIGHTS OF PROGRESS IN

RESEARCH ON ALLERGY AND

INFECTIOUS DISEASES

1959

Items of Interest on Program Developments and Research Studies
Conducted and Supported by the National Institute of Allergy
and Infectious Diseases

VIRUS DISEASES

HA VIRUSES IMPLICATED IN 20 PERCENT OF RESPIRATORY ILL- NESSES IN SPECIAL POPULATION

A recently completed study of the hemadsorption viruses holds them responsible for more respiratory disease among several groups of Washington children than Asian influenza during its epidemic year; points out that 42 percent of the croup cases in the study population were caused by HA viruses; and, tentatively blocks out 20 percent of the childhood respiratory disease total as the province of these newly discovered viruses. Influenza, by contrast, caused only 13 percent of the respiratory diseases in the group under study.

These findings, the investigators caution, reflect only the viral experience of a hospitalized indigent population in the Washington, D. C. area during 1957-1958. In other years and other localities, respiratory illness may be associated with agents inactive during this study.

Results of this broad-scale investigation are reported in the Journal of the American Medical Association by Drs. Robert M. Chanock, M. Katherine Cook, and Albert Z. Kapikian of NIAID's Laboratory of Infectious Diseases; Dr. Thomas Reichelderfer of D. C. General Hospital; and Drs. Robert Parrott, Andrew Vargosko, and Miss Alia Luckey of Children's Hospital. The study population consisted of 1,738 patients from three hospitals in the Washington, D. C. area: Children's Hospital, D. C. General Hospital, and Georgetown University Hospital. These were infants and children brought to the hospital with respiratory illness, plus a group of healthy controls of the same socio-economic background. Infections observed ranged from mild, afebrile illness to pneumonia and croup, the latter

representing the most acute emergency among the various respiratory illnesses of infancy and childhood.

Investigators subjected both patients and controls to the same procedure: a single throat swab taken on admission to the hospital or during a first visit. They compared virus isolation frequency from sick patients against the controls and found Type 1 associated with the sick group 20 times more frequently. Type 2 was associated 3 $\frac{1}{4}$ times more frequently. The former was associated with each of the five diagnostic categories under study: pneumonia, croup, bronchiolitis, pharyngitis, and mild respiratory tract disease. In contrast, Type 2 virus could be related only to croup, pharyngitis, or the mild illness, with the croup syndrome showing the highest rate (16.7 percent) of association.

Virus isolation was supplemented by serologic tests. In patients from whom virus was isolated and from whom paired sera were available, an antibody rise to the isolated agent was demonstrated. The authors found the highest incidence of antibody rise (30.2 percent) for Type 2 virus occurred in the croup syndrome. This supports the high rate of virus isolation (16.7 percent) from these patients. Sixteen percent of patients with pharyngitis or bronchitis developed a Type 2 antibody response; the isolation rate from these patients for Type 2 virus was 5.3 percent. Although Type 2 virus was not recovered from patients with bronchiolitis or pharyngitis, approximately 5 percent of these patients developed antibody for Type 2 virus; thus this constitutes the only evidence implicating this agent in these syndromes.

Serologic evidence of Type 1 infection varied from 12 to 18 percent in the patients with pneumonia, croup, bronchiolitis, or pharyngitis; the corresponding virus isolation rates for Type 1 virus in these syndromes varied from 1.3 to 3.7 percent.

The authors point out the serology indicates a higher relationship between virus and disease than virus isolation tests, the latter implicating them in over 6 percent of respiratory diseases, the former reaching nearly 20 percent. Although a true value probably lies between these estimates, previous reliability of serological techniques indicates a figure close to 20 percent as more realistic.

While influenza accounted for 52 percent of respiratory illness during the single month of October 1957, its relative quiescence during other months reduced its average to 13 percent of the respiratory total.

STAPHYLOCOCCAL PNEUMONIAS
DOMINANT COMPLICATION OF ASIAN
INFLUENZA IN 1957-58 PANDEMIC

Grantees of the National
Institute of Allergy and
Infectious Diseases,
Dr. Christopher M. Martin and

associates at Harvard, Boston, and Tufts university medical centers, have confirmed certain predictions made by public health authorities prior to the 1957-58 influenza pandemic. Studies by the NIAID grantees reported in two articles in the JAMA Archives of Internal Medicine reemphasize that pregnant women and people with chronic heart disease or chronic respiratory insufficiency are particularly susceptible to severe influenzal disease. Therefore, they are prime candidates for immunization. Antecedent conditions in 32 influenza-associated deaths examined by the Boston area investigator included heart disease in 12 cases; pulmonary disease or respiratory insufficiency, such as bronchial asthma, in 10; diabetes 2; cirrhosis of the liver 2; pregnancy 4; miscellaneous 4; and no predisposing condition in 8. (Eight patients had more than 1 antecedent disease.)

Strains of Asian influenza A virus were isolated from tissues of 44 percent of the fatal cases and, by means of fluorescent antibody techniques, influenza A viral antigen was demonstrated in interstitial macrophages of respiratory tract tissues in 31 percent.

Influenzal and post-influenzal pneumonias were the more common and severe complications. Myocarditis was infrequent and generally minimal. The investigators suggest that in times of epidemic influenza, and in areas where staphylococcal infections are highly prevalent, cases presenting signs usually associated with fatal influenza should be treated promptly as bacterial--particularly staphylococcal--pneumonias. Potentially fatal disease, they say, is indicated by the occurrence of hemoptysis, tachypnea and cyanosis in influenza, with or without prominent pulmonary findings.

While the authors decry indiscriminate use of antibiotics, they point out that the interval between the time severe disease was first recognized and the time when death occurred was 48 hours or less in 25 (81 percent) of 31 cases. Most laboratory diagnostic techniques were not rapid enough to be useful; therefore, the opportunity for successful specific treatment was probably missed in some of the cases of bacterial pneumonia. However, even antibiotic regimens will not help in some cases. The most striking characteristic of the organisms associated with both the fatal and the nonfatal cases was the exceptionally high incidence of strains resistant to multiple antibiotics--staphylococcus strains hitherto associated with hospital-acquired infections, but in these studies most often community-acquired. The data show that in the Boston area

penicillin, streptomycin, and tetracycline can no longer be relied upon as effective agents in the treatment of post-influenzal staphylococcal pneumonia. That 6 of the 20 strains of staphylococci tested were resistant to erythromycin suggests that this drug, in general use less than 5 years, may soon join the list of ineffective drugs.

The NIAID grantees comment that the pandemic of Asian influenza A of 1957-58 was the first in which, in nearly all parts of the world, the staphylococci played a dominant rôle in the complicating pneumonias. The fatal cases of staphylococcal pneumonia observed in their studies were generally marked by severe and more rapidly progressing illness, less accurate diagnosis, and relatively ineffective antibiotic therapy.

The relative futility of standard, vigorous supportive measures in preventing eventual asphyxia in the cases reported underscored the need for a practical "artificial lung."

COMMERCIAL VACCINE AGAINST
ASIAN INFLUENZA PROVED HIGHLY
EFFICACIOUS IN NAVAL RECRUITS

A commercially prepared vaccine "provided a high degree of protection against Asian influenza" among Naval

recruits at Great Lakes Naval Training Station according to a joint study made by the U. S. Navy and the National Institutes of Health. The authors of the report which appeared in the New England Journal of Medicine, are Dr. Benjamin F. Gundelfinger and W. T. Stille of the United States Navy; and Dr. Joseph A. Bell of NIAID's Laboratory of Infectious Diseases.

In their study the authors sought to determine the effectiveness of a commercially prepared inactivated monovalent Asian-strain influenza vaccine. They also considered it desirable to determine whether a polyvalent influenza vaccine in prior use by the Military and not containing Asian-strain antigens would provide any protection against or modification of this disease.

A total of 3,355 vaccinated and control recruits who entered training between August 7 and September 18, just prior to the outbreak of the epidemic of Asian influenza, made up the study population. Thirty percent of the recruits were given a single inoculation (200 CCA units) of the monovalent Asian-strain influenza vaccine; 30 percent, a single inoculation (200 CCA units) of the polyvalent influenza vaccine containing no Asian strain; and the remaining 40 percent were given a placebo.

The authors demonstrated Asian influenza infections by both virus isolation and serologic tests throughout the period of study which ended November 9. They obtained paired serum specimens from recruit patients of the control group who were hospitalized for respiratory illness and titrated them by the complement-fixation test to the Asian strain of influenza virus. The pairs showing four-fold or greater titer rises increased from 54 percent early in September to 100 percent for the first week of October. Influenza viruses antigenically similar to Asian strains were isolated from throat specimens of men who showed rises in influenza antibody titer.

In analyzing the data relating to the occurrence of febrile respiratory disease during the epidemic, the authors found the over-all attack rate for those vaccinated with the Asian strain influenza vaccine to be 19.4 per thousand per week; for the polyvalent non-Asian strain influenza vaccine, 90 per thousand per week; and for the controls 113.3 per thousand per week.

In hospitalized cases, perhaps the more severe illnesses, the attack rates were 7.4, 39.0 and 72.4 respectively. Calculating effectiveness in relation to controls, it appears that the Asian-strain vaccine was 83 to 90 percent effective and the polyvalent vaccine, 21 to 46 percent effective in controlling Asian influenza.

This study clearly shows that in practical use a single dose of monovalent Asian strain influenza vaccine gave good protection in adults against clinical influenza during an epidemic caused by the Asian-strain virus. During the past 10-15 years many well-controlled influenza vaccine studies have shown that influenza vaccine gave good protection but in some studies, the vaccine gave little or no protection. It has been deduced that the failures were due to the fact that the epidemic was caused by a virus strain different from that included in the vaccine.

This study is important in that it demonstrates in a single, well-designed and well-controlled study that good protection resulted when the vaccine contained strains antigenically similar to the epidemic strain, and that poor protection resulted when the vaccine strain was not similar. This study also demonstrates that influenza vaccines not containing the epidemic strain gave slight protection.

INFLUENZA OUTBREAKS STUDIED IN ISOLATED BERING SEA COMMUNITIES

The populations of each of these two islands experienced outbreaks of respiratory disease during the fall of 1957. In each instance it was possible to identify the source

of infection. The outbreaks were explosive in character and lasted for a period of only three weeks, at which time all susceptible individuals had become infected. 82 percent of residents on St. Paul Island and 87 percent of residents on St. Lawrence Island developed symptoms characteristic of influenza. These are unusual communities in that there is very close contact between households during the individuals' various activities, which probably accounts for the rapid and wide distribution of Asian influenza virus.

Antibody surveys of serums collected in the areas indicated that there had been no previous experience with swine influenza virus, but there was evidence that there had been widespread infection due to both influenza A and A prime virus in the past. Apparently morbidity from the Asian influenza outbreak was not affected by antibodies present from previous influenza infections.

The disease was generally mild, but lung complications were frequent, and two deaths were associated with the outbreaks.

This study again emphasizes the information that can be obtained from studies of infectious diseases in remote communities where it is possible to investigate outbreaks essentially unassociated with other illnesses. This study by Dr. Robert N. Philip and associates of the NIAID Rocky Mountain Laboratory was published in Public Health Reports.

MEASLES ATTACK RATE REDUCED
3-FOLD BY INOCULATION WITH
CANINE DISTEMPER VACCINE

Association of a canine distemper vaccine with a three-fold reduction in the attack rate of measles is reported

by grantees of the National Institute of Allergy and Infectious Diseases.

Dr. John M. Adams and associates of the U. C. L. A. School of Medicine report their findings in Virology. Their study group consisted of about 2,000 patients at a California state hospital for the mentally retarded. The investigators inoculated 200 subcutaneously with 1 ml. of a vaccine prepared from live canine-distemper virus attenuated by repeated passage through chick embryos. They gave 200 subjects an influenza vaccine; 200, a mumps vaccine; and studied the remainder as controls.

The main objective of the investigation was to determine whether distemper vaccine might reduce the incidence of respiratory infection. This was suggested to the scientists by an observation that distemper antibodies were almost universally present in human serum and by other evidence implying a relationship to some of the common respiratory pathogens.

Recently, however, Adams and Imagawa reported in a separate paper a pathologic and immunologic relationship between measles and distemper. This, along with a measles epidemic experienced by the hospital in 1955, prompted them to evaluate further the results of their previous immunization study.

At the time of the epidemic, 165 of the distemper-vaccinated subjects were still in residence at the hospital. During their re-evaluation the investigators found that only 3 (1.8 percent) of these had suffered infection. Surprisingly, however, the attack rate climbed to 5.85 percent among the 1,519 members of the control group.

While cautioning that the significance of their report is not established, the scientists point out that the first challenge experience of these patients occurred almost 3 years after the administration of only a single inoculation. Furthermore, their data is supported indirectly by the work of a Swedish investigator who reported that distemper antibody titers increased in a study population following a measles epidemic. Finally, the size of the control group--over 1,500 subjects--adds further support to the correlation.

To extend their study, the scientists are now conducting a large-scale field trial of the vaccine in the Los Angeles area. Unlike the earlier investigation, in which only a single inoculation was used, they are administering a series of three, the first two given 1 week apart, the last, 6 months later. Thus far, they report no unfavorable side reactions.

MUMPS EPIDEMIC IN ESKIMOS OFFERS UNIQUE CHANCE TO STUDY DISEASE IN ISOLATED GROUP

Our understanding of epidemic diseases rests to a great extent upon observations made on populations which had not previously been exposed to the specific agent responsible for the infection. Today it is very difficult to study epidemics in such populations, but the opportunity arose to investigate an outbreak of mumps in a "virgin" population of Eskimos in 1957.

The work by R. N. Philip, K. R. Reinhard, and D. B. Lackman of the National Institute of Allergy and Infectious Diseases Rocky Mountain Laboratory in Hamilton, Montana, is reported in the American Journal of Hygiene.

A total of 88 percent of 561 Eskimos developed mumps during the first 6 months of 1957 and of these 27 percent were inapparent infections. In one of the two St. Lawrence Island communities the disease was introduced by a single individual and the resultant outbreak continued over a period of 27 weeks,

while in the other town a number of National Guardsmen returning from an encampment introduced the disease and as a result the outbreak was more explosive and lasted only 13 weeks. There was less disease in very young children and in older individuals than in members of other age groups. Greater inherent resistance and less intense exposure probably accounted for the fewer cases in children and age-associated inherent insusceptibility for the relative lack of cases among older persons. Orchitis occurred in 25 percent of males with mumps. As nearly 40 percent of these had bilateral involvement, it was estimated that 5 or 6 males were rendered sterile as a result of infection. While the frequency of infection was lower in pregnant women, it was significant that infection during the first trimester of pregnancy increased the risk of abortion. Congenital malformations were not encountered.

Complement-fixation tests were made to confirm the diagnosis of mumps. It was found that this test was not effective for the diagnosis of past infections but was extremely valuable in studying recent illnesses.

In view of the disruption of "virgin" communities caused by introduction of mumps and of the seriousness of the disease in causing sterility in males and inducing abortions in pregnant females, administration of vaccines to remote populations seems warranted. The need for continued research into problems which will be encountered by these people as their state is subjected to greater exposure to previously unknown diseases through increased population and transportation is shown by these investigations.

IMPROVED METHODS FOR
STUDYING ENCEPHALITIS
CITED AT SYMPOSIUM

The 1959 outbreaks of Eastern equine encephalitis (EEE) in the New Jersey area (20 deaths, plus complications in non-fatal cases, among 29 diagnosed) heightened interest in the Symposium on Eastern Equine Encephalomyelitis held last November by the University of Maryland at College Park.

Dr. William L. Pond of NIAID's Laboratory of Tropical Virology, emphasized the relative facility with which EEE may now be studied. Only a few days are now required to isolate the virus in tissue culture or in young chicks and to identify it. This research advantage should be exploited, he said. The EEE virus is only one of more than 120 arthropod-borne agents sometimes seen during sporadic epidemics in the temperate zones. These agents are continually active and cause many problems in tropical areas. Virus diseases in this group, including for the most part infections transmitted by mosquitoes (yellow fever, dengue, EEE, for examples) and also by ticks and other

arthropods, represent one of the least explored areas of medical research. NIAID tropical virology investigators here and at the Middle America Research Unit in the Panama Canal Zone are conducting a number of their studies in this field.

The fact that the agents thus far classified are pathogenic in animals suggests that many arthropod-borne viruses may eventually be related to now-undifferentiated human diseases. Recent outbreaks of an arthritic-like disease in Australia, for example, have been related to one of the group. Dr. Pond pointed out that the arthropod-borne viruses are the largest group of infectious agents under one heading and that there appear to be particularly wide variations in pathogenicity among them.

ENCEPHALITIS-INFECTED GARTER-SNAKES PROVIDE FIRST EVIDENCE OF VIREMIA IN SPECIES

The overwintering mechanism of western equine encephalomyelitis is one of the major unsolved problems in the ecology of this

arthropod-borne viral disease. The mosquito-bird-mosquito cycle for disseminating WEE virus in summer in temperate zones is well established, but the paucity of evidence that birds serve as a winter reservoir of virus or reintroduce it into northern areas each year suggests the need for investigation of other possible reservoirs, such as hibernating animals. Rocky Mountain Laboratory scientists became interested in testing the susceptibility of garter snakes to WEE virus when they observed in field studies that Culex tarsalis, mosquito vector of this virus, overwinters in rock piles where many snakes also hibernate.

The RML investigators (Leo A. Thomas, Carl M. Eklund, and William A. Rush) in a preliminary report in the Proceedings of the Society for Experimental Biology and Medicine show that garter snakes can be infected readily with WEE virus either by bites of infected C. tarsalis or by intraperitoneal inoculation of the virus, and the resulting viremia is of high titer and long duration.

In these studies, all of the snakes were blood-tested for WEE virus before infective feeding or inoculation.

In the transmission tests, the C. tarsalis mosquitoes were infected by feeding on 1-day-old chicks which had been inoculated with mouse brain suspension of WEE virus. After sufficient time had elapsed for virus to proliferate, the mosquitoes were fed on garter snakes. Viremia of 4 to 20 days' duration was observed. In one snake, virus was detected in a 10^{-6} dilution of whole blood, which was the highest dilution tested. All of the snakes died between the 8th and 23rd day after

mosquitoes had fed on them, but there was no implication that WEE virus was responsible for death.

In the four snakes injected intraperitoneally with a suspension of the virus, virus was detected in the blood of each snake and persisted in one snake for 36 days, at which time the snake died.

Neutralization tests demonstrated the virus isolated from five of the snakes to be WEE.

These studies, Thomas and his associates say, present the first evidence, to the best of their knowledge, that a virus which is an important parasite of avian and mammalian hosts can infect a cold-blooded vertebrate and cause viremia of high titer and long duration.

STANDARD METHODS DEVELOPED TO DETECT AND QUANTITATE MOUSE POLYOMA VIRUS

As a contribution to research on viruses and cancer--and in line with an over-all objective of characterizing

important mammalian viruses--investigators of the National Institute of Allergy and Infectious Diseases are elucidating the natural history of the polyoma virus. Their initial findings are reported in three articles in the Journal of Experimental Medicine by Isadore Brodsky, John E. Estes, Janet W. Hartley, Robert J. Huebner, William T. Lane, Wallace P. Rowe, NIAID Laboratory of Infectious Diseases and Dr. Lloyd W. Law, NCI Laboratory of Biology.

1. Quantitating and detecting the virus. In developing standard methods the NIAID virologists compared three procedures for usefulness in titration and detection of this agent: production of cytopathic effect (CPE) in mouse embryo tissue culture; production of HI antibodies after inoculation into weanling mice--the mouse antibody production test; and production of tumors in suckling hamsters during a 3- to 5-week observation period.

The CPE and MAP tests were generally comparable in sensitivity, reproducibility, and time required to obtain results. Titration by tumor production in suckling hamsters was not suitable for quantitation because of marked variation in susceptibility among animals. Use of both CPE and MAP procedures gave maximum sensitivity in virus isolation tests. For example, the risk of a false positive appeared minimal with the MAP test in view of the rarity of antibody in control mice held 3 to 6 weeks. ("NIH General purpose" non-inbred Swiss strain yielded no polyoma antibody in more than 1,100 adult mice tested.) This is an advantage over the CPE test, in which the number of nutrient fluid changes required by tissue cultures during long observation tests (14-32 days) and the extraordinary

stability of the polyoma virus make cross-contamination a distinct risk. On the other hand, the CPE test offers an advantage in yielding strains of virus for study--genetic and serologic variants of polyoma virus which would presumably be missed in MAP tests.

2. Virus stability. The mouse polyoma virus was stored at 4° and -60°C. for 8 weeks without any loss of hemagglutination or infectivity titer; storage at 37° for 8 weeks reduced infectivity titer by approximately 2.5 log₁₀ units. Repeated freezing and thawing of infectious tissue culture fluid had no effect on virus titer. Heating the virus for 30 minutes at 60°C. had no effect on infectivity and hemagglutination titers, although at 65°C. there was some loss. The virus was resistant to ultraviolet irradiation and to exposure to 2 percent phenol and 50 percent ethyl alcohol. Other tests also showed that the mouse polyoma agent has extraordinary stability.

3. Distribution of antibodies in laboratory mouse colonies. Eight mouse colonies in Bethesda and elsewhere were surveyed to measure the prevalence and incidence of polyoma infection in various stocks. The mice used as test samples were uninoculated animals never in cage contact with animals inoculated with any material. Frequency of HI antibody varied from 0 to 84 percent in adult mice in different colonies. Antibody was infrequent in mice less than 3 months of age and increased in frequency with age. There was no evidence that infection was specific for particular mouse strains. All tests on 1146 animals in each of the 19 strains examined from the breeding stock in the DRS Animal Production Colony showed no evidence of infection with polyoma virus.

The highest frequency of infection was found in colonies in which breeding mice were housed in proximity to mice inoculated with polyoma virus or passage tumors. Mice from a colony free of antibody became infected when held in room or cage contact with virus-inoculated mice, but at very low rates except in mothers of inoculated litters. The investigators interpret these results as indicating that artificial contamination of the environment is an important factor in determining the prevalence of infection in the colonies observed.

A lack of correlation of polyoma antibody with occurrence of leukemia in AK strain mice suggests that polyoma virus has little or no etiologic relation to spontaneous AK leukemias.

The finding that certain mouse colonies are essentially free of polyoma virus carries several practical implications. Availability of mice known to be free of infection is obviously of great value to the laboratory studying the virus. Also, a study of spontaneous tumors in a polyoma-free colony could

simply and conclusively rule out certain types of neoplasm as being caused by natural polyoma virus infection. In view of the wide pathogenic potentialities of the agent, knowledge of the level of infection in colonies of mice used for experimental cancer work would appear to be of great importance, the investigators note.

A discussion by Dr. Rowe of these quantitative studies of the mouse polyoma virus was presented at the annual meeting of the American Society for Clinical Investigation this year, and has been abstracted in the Journal of Clinical Investigation.

"Mouse polyoma virus can be studied readily by standard virologic procedures, and consequently provides an exceptional model for study of viral neoplasia in a mammal..... Certain of the concepts obtained from the study may be directly pertinent to searches for comparable agents of human tumors."

The polyoma virus, first described by Dr. Ludwig Gross of the VA, has since been extensively studied by Dr. Sarah E. Stewart of NCI and Dr. Bernice Eddy of DBS.

BITE OF INSECTIVOROUS BATS
SHOWN IN LAB TO TRANSMIT
RABIES TO SUCKLING MICE

A bat that attacked hunters in the Bitterroot Mountains of Montana was captured and brought to the Rocky Mountain

Laboratory of the National Institute of Allergy and Infectious Diseases where it was allowed to bite 3 of a litter of 6 suckling mice. The mice which were bitten died in about 2 weeks, and virus was demonstrated in their tissues; the mice which were not bitten remained normal. By means of specific neutralization tests, rabies virus was shown to be responsible for the death of the mice. The bat died before it had an opportunity to bite other animals, but virus was demonstrated in the brain and salivary glands.

By the use of the relatively simple procedure of allowing bats to bite suckling mice, it is possible to answer certain questions of importance in the study of bat rabies, points out Dr. J. Frederick Bell of RML.

It has not been difficult to demonstrate rabies virus in the salivary glands of insectivorous bats, but until this experiment there has been no direct evidence that they were capable of transmitting the virus by bite. This isolation confirms the supposition that bite may be the method whereby virus is maintained in bat colonies and also that rabies in man, as suggested by reports of 3 cases, may result from bites of infected bats.

A total of 11 infected bats of 6 species has been found in Western Montana since 1954. It is interesting to conjecture

as to the role that infected bats may play in initiating outbreaks of rabies in both wild and domesticated animals. For instance, in Montana in 1950 there was an outbreak of rabies in small animals. This was the first recognized outbreak that had occurred in the state for many years and no evidence could be accumulated to suggest how the virus had been imported. It is possible that virus could have been introduced by means of bats rather than by accepted classical methods such as introduction by rabid dogs or cats.

SPECIFIC TISSUE MAY BE
EXTRANEURAL STORAGE
SITE OF RABIES VIRUS

Recent investigations by
Drs. S. Edward Sulkin,
Philip H. Krutzsch and
associates of the University

of Texas Southwestern Medical School suggest that brown adipose tissue is the means by which certain animals serve as reservoirs for the rabies agent in nature. Their work was supported by a research grant from the National Institute of Allergy and Infectious Diseases and published in the Journal of Experimental Medicine.

The investigators comment that despite prolonged and intensive studies of this disease little is known about its survival mechanism in nature. Evidence is now accumulating that the disease may not be invariably fatal once symptoms become established, and many animals including man may be subject to subclinical rabies. In planning this study of the progress of rabies infection in experimentally infected bats, the investigators assumed that those tissues providing food reserve during the state of hibernation might also provide a means for virus storage. They placed special emphasis on the role of the interscapular brown fat since it is an actively metabolizing tissue almost certainly serving as a major food source during winter sleep. Two species, the Mexican free-tailed bat, a quasi hibernator, and the little brown myotis, a true hibernator, were used to locate anatomical sites of rabies virus multiplication.

Because of the difficulties inherent in working with winged animals, the Syrian hamster, a hibernator highly susceptible to rabies infection, was also selected to provide a more effective means for defining the role of brown adipose tissue in the pathogenesis of rabies. In both an experimentally infected little brown myotis and in the Syrian hamster, rabies virus was isolated more frequently from the brown fat than from the salivary gland indicating that, at least in the susceptible host, brown adipose tissue may be as frequent a site of viral proliferation as the salivary gland.

The investigators isolated rabies virus from the brown fat tissue of experimentally infected animals with frequencies

ranging from 22 percent in the relatively insusceptible Mexican free-tailed bat to almost 62 percent in the Syrian hamster. Quantitative assays of infected brown fat tissues revealed titers equal to the mouse i.c. LD 50 titer of the rabies virus inoculum, indicating active multiplication of the virus.

The investigators suggest the possibility that similar mechanisms may be involved in the maintenance of other viral agents during interepidemic periods. As one example, the fact that Western equine encephalomyelitis virus may infect garter snakes suggests that in this cold-blooded animal there may be a tissue serving in a similar manner to the "hibernating gland" of warm-blooded animals as a storage site for virus during the winter months. The investigators are now proceeding with further studies of which preliminary results already implicate the brown adipose tissue in bats in the pathogenesis of certain arbor viruses.

TOBACCO MOSAIC VIRUS MAY
PROVIDE CLUES TO FUNDAMENTAL
CHEMISTRY OF MUTATION

Study of mutations of the tobacco mosaic virus (TMV) "transcends the implications it has for virology," and may

provide clues to genetic mechanisms fundamental to living systems, according to Dr. C. A. Knight of the University of California. His study of the TMV-variant system is supported by grants from the National Institute of Allergy and Infectious Diseases and the Rockefeller Foundation.

An extensive background of experimental data on TMV is available to Dr. Knight. First virus discovered (Iwanowski, 1892), this agent has been studied intensively at the University of California virus laboratories headed by Dr. Wendell Stanley. Dr. Stanley, a Nobel-Prize winner, was the first to crystallize a virus (TMV) in 1935, demonstrating it to be a nucleoprotein essentially similar to nuclear constituents of typical cells.

A virus differs from known living organisms in being a definite chemical compound rather than a complex mixture of compounds. This relative simplicity may be an advantage in elucidating the chemistry of mutation.

A single particle of the rod-shaped TMV measures about 15 by 300 millimicrons (1/250,000 inch). The most detailed information on TMV structure has been obtained from X-ray diffraction. Patterns of X-rays passing between the atoms of a virus provide clues to structure. The TMV particle, suggests Dr. Knight, may be considered as a giant molecule with its approximately 2100 subunits spiraling in a helical pattern around a 300 angstrom (1/250,000,000 inch) axis. Structurally, the virus strains differ primarily in the density of the protein lying at particular radii.

The subunits contain about 164 amino acids. Thus, the investigator points out, it will be a major task to find complete sequences of these. However, a clue to similarities and differences is being obtained by tryptic digestion of the strain proteins. The results suggest that differences tend to be localized and considerable segments of protein chains are probably identical.

A beginning has been made in investigating the other major constituent of the TMV particle, namely the ribonucleic acid (RNA). Only the pyrimidine mononucleotides have been quantitatively determined thus far. Clearly different amounts of these were released from one of the TMV strains examined, providing the first evidence of structural differences between strain nucleic acids.

In summary, Dr. Knight comments that there are apparently great similarities in the chemical structure of strains of TMV, but there are distinctive differences. The specific manner in which these findings can be related to the biological properties of the viral strains as an index of the chemistry of mutation is an area which is just beginning to be explored.

The report was published in the proceedings of the Fourth International Congress of Biochemistry.

RODENTS PROVED RESERVOIR FOR COLORADO TICK FEVER VIRUS IN NATURE

Adult ticks transmit CTF virus to man but the incidence of infected ticks varies tremendously from one small

area to another. In 5 areas under study by the Hamilton, Montana, Rocky Mountain Laboratory of the National Institute of Allergy and Infectious Diseases, the percentage of infected adult ticks varied from 0 to 16 percent. RML investigators Willy Burgdorfer and Carl M. Eklund report in the American Journal of Hygiene that such variations appeared to be due to the degree of infection in the rodents in the area. Among Columbian ground squirrels there were 27 of 209 (13 percent) infected. The infection rate varied from 0 to 27 percent in the various areas. In the area in which no ticks were infected no disease was demonstrable in Columbian ground squirrels. Among the golden-mantled ground squirrels, virus was detected in 28 of 61 specimens (45.9 percent). These animals were abundant only in the areas in which the number of infected adult ticks was high. The latter squirrel is the preferred host of immature stages of the tick. It was demonstrated that animals in nature may circulate virus for a period of at least 20 days.

The fact that there is a persistent viremia in animals that are fed upon by both larval and nymphal ticks may be significant in the ecology of CTF virus, for an animal may be infected by a single nymph and subsequently infect large numbers of larvae. These larvae would then constitute a source of infection the following year.

On the basis of studies performed in Colorado and Montana, it is apparent that the local rodent population is responsible for the maintenance of CTF in nature and thus for its spread to man. The significant rodent host may vary from area to area and each local health unit must determine this fact before effective biological control may be applied.

ALLERGY-IMMUNOLOGY

STRESS SIGNIFICANT FACTOR IN RESISTANCE TO PASSIVE ANAPHYLACTIC SHOCK

Immediately following periods of stress, mice are less susceptible to passive anaphylactic shock than their unstressed counterparts according to Dr. A. F. Rasmussen, Jr., Edwin S. Spencer and James T. Marsh, grantees of the National Institute of Allergy and Infectious Diseases. Having observed a similar diminution of susceptibility following the administration of cortisone, they theorize that increased adrenal cortical activity may be responsible. The investigation was reported in Proceedings of the Society for Experimental Biology and Medicine.

As part of a larger study evaluating the nature of host response to shuttlebox stress, the investigators tested mice for susceptibility to anaphylaxis following stress. During the course of the study, they subjected 4-5 week-old mice to avoidance-learning stress in shuttleboxes for 6 hours a day for periods of 1 day or 4 weeks. Immediately following the last period of stress, each mouse was injected with .5 ml. of a mixture of equal parts of undiluted anti-BPA mouse serum and 2 percent BPA, previously established as the most effective shocking dose.

The scientists scored shock on a relative basis ranging from fatal through severe to moderate, mild, and no signs of shock.

In one series of experiments fatal shock occurred in 17/26 controls. In contrast to this, only 2 out of 26 in the combined stressed groups expired.

In a second series, the investigators subjected mice to avoidance-learning stress for a single period of 6 hours and then tested for susceptibility to passive anaphylaxis in the

same manner as previously described. In the first of 2 experiments 8 of 8, and in the second 7 of 9 stressed mice survived shocking doses of BPA and BPA antiserum lethal to 8 of 10 controls in the first and all of 18 controls in the second experiment. While many of the survivors exhibited mild to moderate shock, it was pointed out that differences in severity scores were highly significant.

The investigators conclude that whatever the finally determined mechanism, this change in susceptibility provides additional evidence for the influence of environmental stress on host response to infections and allergic disease.

GIANT RAGWEED POLLEN PURIFICATION SOUGHT BY COMMITTEE ON STANDARDIZATION OF ALLERGENS

The new, full-time Committee on Standardization of Allergens under the chairmanship of Dr. Dan Campbell of the

California Institute of Technology held its first research conference at the National Institutes of Health in June 1959. The Committee discussed in detail the initial practical steps that must be taken in the proposed program of studies to devise a practical method for ragweed pollen purification.

For some 30 years, allergists have designated allergen standardization the single most important consideration in the field of clinical allergy. Any further advance in this area of immunological research is generally considered unlikely until the scientific investigator may consistently provide himself with allergenic products which meet rigid norms of potency, purity and specificity. At present, the allergist is frequently unable to demonstrate a specific irritant in patients undergoing tests, since most allergenic extracts in use are crude preparations composed of several components related and unrelated to the specific sensitization under investigation.

Aware that newer purification techniques like paper chromatography seem to promise progress toward a solution of the problem, the Council had earlier appointed an ad hoc committee to discuss present activities in this area and to consider future plans.

At a recent meeting, the Committee was informed that three investigators--Dr. A. R. Goldfarb, Chicago Medical School; Dr. Bram Rose, McGill University, Montreal; and Dr. Einer Hammarsten, Karolinska Institute, Stockholm--employing dissimilar chemico-physical methods and working independently, have obtained highly purified and potent fractions of ragweed, the most important single allergen in this country. These fractions, although not yet compared, were assumed to be similar in constitution. As a result, the committee

recommended to the Council that it provide funds to interested scientific investigators for characterization and comparison of the three products in order to confirm their equivalence and--if possible--to create a template for future investigations using other allergens.

The Council then established a continuing committee under the chairmanship of Dr. Campbell. When the program reaches its proper stage of development, Dr. Campbell is authorized to expand the base of his permanent committee to include representatives of industry and other Government agencies who may be concerned.

PURIFIED HOUSE DUST
ALLERGEN FRACTION ISO-
LATED AND CHARACTERIZED

The importance of house dust in clinical allergy has been appreciated since Cook's demonstration in 1922 that

this disreputable conglomerate contained substances which could produce intense skin reactions in some asthmatics. House dust has since proved a frequent cause of sensitization. About one-third of all allergic individuals give a positive skin reaction to house dust extracts.

Dr. Wilton E. Vannier of the National Institute of Allergy and Infectious Diseases, Laboratory of Immunology, is working at the California Institute of Technology with NIAID grantee Dr. Dan H. Campbell, who in recent years has studied various polysaccharide components of house dust. This research was also partially supported by the American Academy of Allergy.

Current studies, reported in the Journal of Allergy, are concerned with a purified skin reactive polysaccharide fraction isolated from a pool of house dust samples and mattress dust supplied by a cleaning establishment. The fraction was found to be a heterogeneous mixture of acidic polysaccharides of the following approximate composition: 5 percent polypeptide and 95 percent polysaccharide, containing about equal amounts of uronic acid (probably glucuronic acid), D-glucose, D-galactose, D-mannose with lesser amounts of L-rhamnose and L-arabinose. The present data do not permit a decision as to whether the dust allergen specificity is associated with the polysaccharide or polypeptide portions of the dust fraction. The purified dust extract shows skin reactivity with most, but not all, individuals sensitive to less purified preparations, suggesting the presence of more than one allergen specificity.

The investigators hypothesize that the heterogeneous mixture of materials obtained in dust extract fractions may originate by some sort of degradation process, involving air oxidation with or without the action of bacteria or molds, of a preformed

large polysaccharide or polysaccharide-polypeptide polymer. They comment that even though the problem of the chemical nature of the specific house dust allergen has not yet been solved, these studies have revealed new information about the nature of materials used clinically for the past 30 years.

METHOD DEvised TO INCREASE
YIELD OF POTENT ANTIBODIES
IN ASCITIC FLUID

A method for consistent
production in mice of large
amounts of ascitic fluid
containing various antibodies

of high potency has been demonstrated by the Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases.

Rose Lieberman, John C. A. Douglas and William Humphrey, Jr. of NIAID reported this work briefly in Science. In more extensive papers presented in part at the April Federation meetings the NIAID investigators, aided by Nathan Mantel, National Cancer Institute statistician, enlarged upon this work and also described certain of the immunological, physical and chemical properties of the ascitic fluid.

Procurement of large amounts of potent antibodies from mice has presented difficulties. The sacrifice of large numbers of immunized mice limits their further use experimentally. Investigations of methods for providing sources of abundant quantities of potent antibodies that could be utilized repeatedly were initiated.

The NIAID method consistently produced potent ascitic fluid in 100 percent of mice. In one experiment, two to three intraperitoneal inoculations per mouse of 0.4 to 0.5 ml. of treated suspensions of Staphylococcus aureus, strain 18 and strain 2, or Salmonella enteritidis, mixed with Freund's adjuvant and given at 5-day intervals, produced ascites in all mice tested.

The investigators point out that the usefulness of the technique lies in the fact that one may employ various antigens to produce high levels of antibody in the blood. Subsequently, when ascitic fluid is induced by staphylococcus, for example, the other type antibodies will spill over into and be abundantly available in this ascitic fluid.

In various experiments, 200 mice were employed and received different volumes and schedules of antigen. A very definite relationship between volume of dose and time between inoculations was found. The maximum yields were obtained at high dose level with inoculations spaced closely together.

Although paraffin oil adjuvant alone was capable of eliciting ascites in a significant number of mice, this was not a consistent phenomenon. On the other hand, the staphylococcus and salmonella antigens, although incapable of inducing ascites alone, apparently stimulated the production of this fluid by the adjuvant and consistently affected 100 percent of the mice tested in some experiments, and nearly 100 percent in all experiments (involving over 1,000 mice).

Four different strains of mice, including NIH general-purpose Swiss Stock, were used and all gave comparable results except the A/HeN strain, which gave a low yield.

Histologic sections of peritoneal granulomas and lymph nodes from ascitic mice indicated the presence of large numbers of plasma cells. The relationship of these cells to high titers of antibodies and high levels of gamma globulin bears further study.

The investigators also report intensive research on some of the immunochemical, physical and chemical properties of the ascitic fluid. They demonstrated the presence of high levels of agglutinating antibody for S. aureus appearing early and persisting for more than 100 days in ascitic fluid. Comparable titers were also demonstrated in the serum. The ability of the ascitic fluid actually to confer passive protection was demonstrated in the case of S. enteriditis. Only 0.01 ml. protected over 50 percent of mice.

The results obtained have a number of implications for useful employment. Both protective and agglutinating titers of antibodies have been demonstrated. Work in progress indicates presence of complement fixing hemagglutination inhibition antibodies. High leucocyte counts found in early ascitic fluid would suggest its usefulness as a source of viable, unclumped leucocytes comparatively free of erythrocytes. Possibly, judicious employment of booster antigen-adjuvant inoculations could maintain leucocyte levels as well as volumes of ascitic fluid. The existence of large numbers of plasma cells in lymph nodes of ascitic mice could be related to the stimulated production of antibodies. Availability of these materials promises to facilitate many types of studies in basic immunology, such as those concerned with cellular immunity, anaphylaxis and allergy in general.

PURIFIED ANTIGEN FROM HISTO-
PLASMOSIS ORGANISM EFFICIENT
IN DIAGNOSING DISEASE

Previous studies have shown that most of the immunologic activity of Histoplasma capsulatum is associated with the cell walls. Present findings published in the Journal of Infectious Diseases are concerned with antigens isolated from

the medium in which organisms were grown and were allowed to autolyze for a period of at least 18 days. The material which is contained in the medium consists probably of the more soluble components of the cell wall and possibly of protoplasm.

RML investigators S. B. Salvin and R. F. Smith report that the antigen which was isolated was one hundred to a thousand times more active than histoplasmin in producing delayed reactions in sensitized guinea pigs and was much more specific than histoplasmin since it usually failed to produce reactions in animals which were infected with the related organism, Blastomyces dermatitidis. When tested in 20 human beings, as little as 10 micrograms of material was sufficient to produce lesions sufficiently large to establish a diagnosis of histoplasmosis. It was as efficient as killed whole cells in producing immunity in animals.

A chemical analysis indicated that at least 3 carbohydrates were present in the antigen and that a protein was firmly attached to the carbohydrate portion. It was not possible to separate the protein from the carbohydrate by chemical means. The intracutaneous activity of the antigen in infected animals is more closely associated with the protein than with the carbohydrate component.

These studies are of interest since they demonstrate that immunologically active antigens may be extracted from cell walls of fungal agents and can be concentrated and purified by appropriate methods. Since this antigen appears to be much more specific than ordinary histoplasmin, it should be useful in establishing the diagnosis of histoplasmosis in man.

BACTERIAL DISEASES

<p>LOWER MORTALITY THRU DRUGS OVERSHADOWED BY INCREASES IN INFECTIONS FORMERLY INNOCUOUS</p>	<p>The favorable impact of the antibacterial drugs in reducing the number of deaths from pneumococcal and the hemolytic streptococcal infections has actually been overshadowed by an increase in deaths from infections by <u>Staphylococcus aureus</u>, the enterococci, and certain coliforms which only rarely caused fatalities before the era of antibacterial drugs.</p>
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This is the conclusion drawn from a study of patient records at Boston City Hospital by Dr. Maxwell Finland and associates of the Harvard Medical School. Their work was supported by a grant from the National Institute of Allergy and Infectious Diseases and reported at the 1959 meeting of the Association of American Physicians at Atlantic City.

Dr. Finland's data fill a long-felt need for more reliable information on the changing pattern of bacterial infections relative to the use of antimicrobial agents. Because of the unwieldy volume of material, the survey was restricted to: 1935--the year before the introduction of sulfanilimide; 1941--before penicillin; 1947--before the "broad spectrum" antibiotics; 1951--before the "anti-staphylococcal" antibiotics and 1953, '55 and '57--to define recent trends.

For comparison, the investigators chose the following categories: autopsies with postmortem cultures, bacteremias, bacterial meningitides and empyemas.

During the 24-year period the number of yearly autopsies increased by about 60 percent but those which yielded Staphylococcus aureus increased some 350 percent--a crude index of its increasing significance as a major cause of death.

Most revealing, the investigators point out, were the trends in mortality figures among patients with blood cultures positive for bacteria other than the pneumococci and streptococci. Enterococcal bacteremias, for example, almost unknown before the antibiotic era accounted for 5 to 13 deaths each year from 1947 on.

The greatest and most significant increase occurred in the cases and the number of deaths from Staphylococcus aureus bacteremia. In the former the investigators found a sharp increase in 1941 and a temporary decline following the early use of penicillin. From then on a steady increase occurred so that in 1957 there were nearly four times as many cases as in 1935 and more than twice as many as in 1947. In the latter instance deaths decreased markedly when penicillin first came into wide use, then increased nearly five-fold between 1947 and 1957.

Equally striking are the death figures among patients with gram-negative bacillemias. The number of fatal cases of E. coli bacteremia increased steadily from 10 in 1935 to 30 in 1953. Since 1947, the number of deaths in patients with aerobacter bacteremia (not even identified in blood cultures until after 1941) have closely approximated those in which E. coli was the blood stream invader. Fatal cases of pseudomonas bacteremia increased steadily from none in 1935 to 13 in 1957. Deaths in patients with Proteus bacteremia increased from 5 in 1941, a pre-antibiotic year, to more than 30 deaths in 1957.

In his presentation, Dr. Finland emphasized that he pursued information only on those hospitalized patients treated

intensively with antibacterial agents. The figures for bacterial meningitides and empyemas reveal fluctuations and less clear-cut trends than those for the bacteremias. Nevertheless, when the four categories are taken as a totality, "The net result has been a drop in the number of deaths from bacterial infections through 1947 followed by a significant and steady increase--beyond that which antedated the era of antibacterial drugs."

SUBSTANTIAL NUMBER OF
HOSPITALIZED CYSTIC FIBROSIS
PATIENTS REVEALED BY SURVEY

In line with the request by a Congressional committee for more accurate statistics on the incidence of cystic

fibrosis, the Children's Bureau and the National Office of Vital Statistics, partly supported by a special grant from the National Institute of Allergy and Infectious Diseases, have completed the first phase of a study designed to reveal the magnitude and characteristics of the health problem presented by this disease.

Results--reported in the Public Health Reports by Dr. Monroe G. Dirken, Dr. Marian M. Crane, Mr. Morton L. Brown and Dr. Elizabeth R. Kramm--were drawn from a survey of the number of children with a diagnosis of cystic fibrosis cared for in hospitals from 1952 through 1958.

The survey, made during a 5-week period early in 1959, covered about 9 percent of all listed hospitals including all those approved for pediatric residency.

Figures show that during 1957, 2,500 cystic fibrosis patients, 95 percent under 20 years of age, were discharged from or died in hospitals--about 1 in 7 or 360 were fatalities.

At this stage of the study figures have not yet been obtained on the ratio of those hospitalized to the total number of patients with the disease, although it is believed the former may represent only a fraction of the total. Dr. Harry Shwachman of the Children's Medical Center in Boston, estimates, for example, that 25 percent of 500 children examined by him have never been hospitalized and Dr. Gordon E. Gibbs of the University of Nebraska observes that only 4 of 40 cystic children under his care have been hospitalized.

The investigators' estimate of hospital deaths is supported by an alternate study undertaken by the National Office of Vital Statistics which coded the disease separately in its analysis of a 10 percent sample of 1958 death certificates. This analysis revealed an estimated 560 deaths, 400 occurring in hospitals.

Dr. Dirken and his associates are now actively engaged in the next phase of the survey, to be concerned primarily with the development and testing of methods for conducting a national epidemiological study of the disease.

NEW RESEARCH ON CYSTIC
FIBROSIS SUBJECT OF FIRST
INTERNATIONAL CONFERENCE

Seventy leading investigators met in Washington, January 7-9, for a research conference on cystic fibrosis, a poorly understood disease of children. The three-day conference attracted specialists from the United States and Europe and was co-sponsored by the National Institute of Arthritis and Metabolic Diseases and the National Institute of Allergy and Infectious Diseases, in collaboration with the National Cystic Fibrosis Research Foundation. Chairman of the conference was Dr. Rustin McIntosh, professor of Pediatrics at Columbia University Medical School.

The conference was designed to suggest and stimulate new research concerning the basic nature of the disorder. It included discussions of possible new avenues of approach to prevention and treatment.

Cystic fibrosis (CF) is a generalized disease of the exocrine glands and has been called a disease of childhood only because its victims seldom lived to adulthood in the past. The major symptoms result from the production of an abnormally thick and sticky mucus which may interfere with normal breathing, digestion or both. In the lungs the thick mucus obstructs the air passages and leads to recurring lung infections. When the pancreas is involved, the mucus often clogs the ducts of the gland, limiting the normal flow of digestive enzymes to the intestine.

Research on the basic nature of the disease has been hampered by the lack of experimental animals that develop the disease and the difficulty in obtaining "pure" samples of the abnormal mucus. The mucoid material obtainable from the lungs or duodenum is always contaminated with cellular debris and other material.

One of the characteristics of the disorder is an abnormally high chloride concentration in both the sweat and tears of CF patients. It was reported at the conference that in some instances relatives of cystic fibrosis patients have also shown abnormal sweat electrolyte patterns although they had no overt symptoms of the disease. This finding supports the general belief that the basis of the disorder is genetic and suggests the possibility that heterozygous carriers of the genetic defect might be identifiable by physiological or biochemical tests.

In discussions of the biochemical aspects of the disease, the greatest emphasis was placed on the reported finding that the normal ratio of fucose and sialic acid in mucus was altered in CF patients. Duodenal mucus from these patients showed relatively higher concentrations of fucose, the increase appearing to be related to the increased viscosity of the CF mucus.

The disease presents an extremely complex problem because it involves abnormalities of a wide variety of organ systems in the body. New areas of research suggested included further study of many physiological factors including circulation and innervation of affected tissues, the use of advanced morphological techniques such as X-ray diffraction and electron microscopy, and the biochemistry of exocrine secretions.

Only one paper was presented on the clinical aspects of the disease. This concerned the study and control of concomitant staphylococcal infections. Data were reviewed showing the effect of continuous antibiotic therapy over a 30-day period following diagnosis. The common pattern is a sharp and rapid drop in the infection rate during the initial 2 weeks, then a return to a point almost equal to the original level. Evidence indicates that these patients acquire antibiotic-resistant strains of staphylococcus sooner than normal children. Despite the persistence of this organism in the respiratory tract of fibrocystic children, such patients have a higher than normal resistance to staph infections elsewhere in the body.

Data reported at the conference indicated that the immunological mechanisms in CF children do not differ from those of normal children. Injected antigens elicit satisfactory antibody response, and patients with this disease show no demonstrable increase in susceptibility to viral infection. Such infections, however, apparently precipitate or pave the way for overwhelming bacterial complications.

TETRACYCLINE THERAPY REDUCES NUMBER AND SEVERITY OF ATTACKS OF BRONCHITIS

Promising results from long-term studies of the broad-spectrum antibiotics have been not only a sharp reduc-

tion in the number of attacks of bronchitis and bronchiectasis following oral administration of tetracycline but also a significantly shorter illness in those who were infected. These findings were reported at a 1959 meeting in Washington by Dr. Harry F. Dowling, Professor of Medicine at the University of Illinois and grantee of the National Institute of Allergy and Infectious Diseases. The one-day symposium, attended by about 400 physicians, was jointly sponsored by the D. C. Academy of General Practice, the Maryland Academy of General Practice, and the American Medical Women's Association, Branch 1.

Over periods ranging from 3 to 36 months, 40 patients at the University of Illinois Research and Educational Hospitals participated in a double blind study which gave results consistently favorable to the tetracycline-treated group. Twenty patients who received placebos had acute infections in the respiratory tract below the pharynx at the rate of 4.7 per patient per year; among the 20 receiving tetracycline the rate was 2.7 per patient per year. The placebo-treated group experienced 43 days of illness as compared to 24.6 for those to whom the drug was given.

Even more striking was the result in illnesses with fever. These averaged 8 days per patient per year in the untreated patients in contrast to 1.2 for those receiving tetracycline. The number of febrile episodes per patient per year was 1.9 for patients who received no drug and 0.3 for those who received it.

No serious side effects were observed among these patients. Gastrointestinal symptoms occurred a little more frequently in the group taking the drug. These symptoms were easily controlled by a temporary cessation of therapy or by a reduction in the prescribed dosage of 0.5 gm four times daily.

Specimens of sputum were obtained monthly before and during therapy. The percentage of pathogenic bacteria in the specimens from those receiving tetracycline markedly decreased. Haemophilus influenzae, present in 19 percent before treatment, decreased to 12 percent; pneumococci decreased from 16 to 5 percent during therapy; coagulase-positive staphylococci decreased from 17 to 3 percent.

While this research was in progress, a study of similar design on a larger group was reported by the British Medical Research Council. One hundred and sixteen patients with bronchiectasis were treated with oxytetracycline or penicillin compared to placebos and the results were essentially similar to those described by Dr. Dowling.

Dr. Dowling's paper will appear in the Medical Annals of the District of Columbia. The material on which it is based has been published in a more extensive version entitled "The Long Term Treatment of Bronchiectasis and Chronic Bronchitis" in the Archives of Internal Medicine.

NEW JAPANESE DRUG, KANAMYCIN,
EXCELLENT AS INTESTINAL
ANTIBIOTIC

Kanamycin has been named the most desirable single agent yet evaluated for preoperative preparation of the colon. It controlled bacterial growth in the colon in superior fashion,

was negligibly absorbed from the intestinal tract, and contributed no undesirable side reactions in the study, says Dr. Isadore Cohn, Jr., of the Louisiana State University School of Medicine.

Dr. Cohn drew this conclusion from an intensive study of 18 patients with no known lesions of the large bowel. His study was supported by the National Institute of Allergy and Infectious Diseases. After taking a control stool from each patient, he gave them kanamycin in an oral dose of 1 gm. every hour for 4 hours, then 1 gm. every 6 hours for 72 hours, the total dose being less than 20 grams. Stools were collected daily during the administration of the drug and for 3 days thereafter.

Dr. Cohn's investigations revealed that kanamycin either completely or almost completely controls the growth of streptococci, coliforms and clostridia. He found staphylococci only infrequently, but these were controlled when present. One major difference between kanamycin and other agents recommended for intestinal antiseptics is its failure to affect significantly the count of *Bacteroides* in approximately half the patients. This may not be a real disadvantage, however, since they are rarely the cause of a major surgical infection.

Most of the organisms remained sensitive to kanamycin after therapy, except that no reliable observations could be made on staphylococcus because of its infrequent appearance. The investigator took stool levels of kanamycin from 12 patients to measure loss of the drug as it passed through the gastrointestinal tract. On the final two days of therapy, he found that all patients had over 1000 micrograms per gram of stool, some of them as high as 12,400 micrograms with levels over 5000 common. In 6 patients, he determined blood levels but found no antibacterial activity, indicating the drug exerts maximum effect along the entire length of the large bowel.

Prothrombin time after therapy did not differ from the control value in any patient.

Dr. Cohn suggests that kanamycin may also be of value in the therapy of diverticulitis, the long-term therapy of cirrhotics or others with severe liver disease, and any other condition where prolonged control of the gastrointestinal flora is desired without systemic antibiotic absorption.

Kanamycin, an antibiotic of the actinomycetes, was discovered in Japan by Dr. Jamao Umezawa and assigned to the species *Streptomyces kanamyceticus*. The strain was a rare one among those found in Dr. Umezawa's screening studies to produce water-soluble, basic antibiotics. It was first reported in the Japanese Journal of Antibiotics in 1957.

Kanamycin is manufactured in this country by Bristol Laboratories under the brand name Kantrex. It is bactericidal against a wide variety of gram-positive and gram-negative organisms, including strains of Micrococcus pyogenes var. aureus.

CATHETER VALVE PREVENTS
URINARY TRACT INFECTION BY
ALLOWING INTERMITTENT DRAIN

It is necessary in the clinical management of many conditions to leave a catheter in place, but urinary tract

infections eventually develop in almost all patients. The pathway of infection from indwelling catheters into the bladder along the thin, fluid-filled space between urethral mucosa and catheter was described by grantees of the National Institute of Allergy and Infectious Diseases at Harvard and Boston City Hospitals in an earlier report. Now these grantees, Dr. Edward H. Kass and associates, have achieved clinical success with a technique for eliminating the bladder infection. Their report appears in the Journal of the American Medical Association.

In this technique for prevention of infections due to indwelling catheters, the bladder is irrigated continuously with 0.25 percent acetic acid (pH 5.0 or less) flowing in through one lumen of a three-lumen catheter at a rate approximately equal to urine production. Other lumens provide for the small balloon that holds the tube in place and for drainage of the acetic acid sterilizing solution and urine.

A constantly empty bladder contracts with discomfort to the patient, so an electromechanical valve has been developed by the grantees to provide intermittent drainage; for example, to open the catheter outlet for five minutes out of each hour. The instrument, consisting of a motor driven timer which actuates a solenoid (electromagnetic) operated clamp, is attached to the bed and is silent. Electrical failure merely allows the valve to remain open.

The technique utilizing this valve was successful in clinical trials on more than 100 patients. Less than 10 percent of patients whose catheters were managed by this method developed high bacteria counts in the urine, whereas 100 percent in a control group developed bacteriuria. Patients have been maintained for as long as 60 days by the new procedure without infection of the bladder. No important difficulties have arisen in the studies thus far, although local sensitivity to the acetic acid solution was found in a few patients.

LIVE VACCINE SIGNIFICANTLY
INCREASES RESISTANCE TO
BRUCELLOSIS IN GOATS

Brucellosis continues to be a major animal-human disease problem. Control of the infection in livestock has been

only partially successful. A vaccine made from strain 19 of Brucella abortus has somewhat reduced the incidence of the disease in cattle, especially when combined with adequate sanitation measures and slaughter of infected animals.

However, more than half of the world's population depend on goats for milk. One-half of all goats are believed infected with Brucella melitensis. No vaccine has been developed effective against this organism.

Working under a grant from the National Institute of Allergy and Infectious Diseases, Sanford S. Elberg of the University of California tested the efficacy of a live vaccine prepared from the Rev I strain of B. melitensis. He reported his study in the Bulletin of the World Health Organization.

Results of preliminary tests on 54 adult goats in the Province of Cordoba, Spain, were evaluated to determine the 50 percent infective dose (ID_{50}) of strain 6015, a virulent challenge strain of B. melitensis, in immunized and non-immunized goats.

The animals were injected with 1×10^9 cells of the vaccine strain subcutaneously in the left suprascapular area. After 12 to 14 weeks, groups of animals were challenged with graded doses of the virulent strain in the right suprascapular area. At the same time non-immunized animals were also infected with graded doses of the challenge strain. During the pre-challenge period the immunized animals were bled at weekly intervals for blood culture and serological tests. Blood samples were taken from the control animals as well, after the challenge dose had been administered. Animals were classified as infected on the basis of a single colony of Brucella isolated from a single lymph node.

The tests suggested that the vaccine increased the resistance of the immunized animals 340 to 12,000 times. Perhaps more significant, however, is the fact that immunity lasted a minimum period of 15 months. Furthermore, within this period it was not possible from the experimental design to show any beneficial effect of a booster dose of vaccine. If the immunity conferred by this vaccine strain acts in a similar way to that conferred by the bovine vaccine strain, and there is no evidence yet that the B. melitensis vaccine strain is different in potency, it is probable that the immunity will last longer than 15 months.

Since the number of experimental animals was small, caution must be exercised in predicting the behavior of the vaccine

under natural conditions. Determination of the actual value of the vaccine in the control of goat brucellosis must await large-scale trial under field conditions.

RELATIONSHIP OF PPLO TO HUMAN
DISEASE EXPLORED AT FIRST
INTERNATIONAL CONGRESS

The first international meeting on pleuropneumonia-like organisms was held January 1959 at the Barbizon-Plaza

Hotel in New York City under the auspices of the New York Academy of Sciences, Section on Biological and Medical Sciences. Attended by 220 scientists from 10 countries, the conference was made possible through an NIAID grant to Dr. Robert L. Burkhardt of the Academy and Dr. John B. Nelson of the Rockefeller Institute.

Relatively few studies have been made of the pleuropneumonia-like organisms (PPLO) since 1898 when the Pasteur Institute in Paris first found the microorganisms in animals. They have continued to pose a threat to livestock and poultry producers, and entire herds of cattle have been destroyed in order to eradicate disease from an area. Following the first isolation from humans by Dienes and Edsall in 1937, these filterable agents have been found to be more widely disseminated and more closely associated with disease than had previously been recognized.

PPLO have been found in several species of mammals and in avian species. They have been isolated from the human genito-urinary tract, alimentary tract, fluid of arthritic joints, eye tissues and skin lesions. Injection of PPLO into rats, mice, and several other species of animal has produced arthritis. Attempts are being made to demonstrate an etiologic role in certain forms of human arthritis. For example, the organisms have been recovered from lesions of Reiter's disease (an affliction of males characterized by initial diarrhea, followed by urethritis), conjunctivitis and migratory polyarthritis frequently accompanied by keratotic (horny) lesions of the skin.

Several grants by NIAID and NIAMD support university research in this area. For example, NIAID grantees Drs. Henry E. Adler, J. E. Moulton, and Richard Yamamoto of the University of California have published reports on PPLO avian strains. In one of their studies, chicken embryos, selected one to eight days after inoculation with PPLO, were observed during the development of arthritic lesions from the earliest stage of hyperemia to the stage of fibrous organization. The NIAMD also has studies of the relationship of this type agent to human arthritis under way or planned at its Bethesda laboratories.

Some of the investigators at the meeting described PPLO as an autonomous and distinct microbial group, characterized by growth into plastic branching filaments and the breaking up of these into granules. Others pointed out that filaments have not been observed in many PPLO strains except in rudimentary form, and suggested that these organisms constitute a different morphologic group, but not a separate class or family. In many ways PPLO resemble the L forms of bacteria, which also grow without a rigid cell wall. The fact that PPLO pass through many bacterial filters had inclined some investigators to class them with viruses.

The conference served to integrate information from various scientific disciplines on the cultural requirements, morphology, biochemistry, serology and pathology of PPLO, and included discussions of a number of species of this agent from avian and mammalian sources. Controversies over the place of PPLO in microbiology and in the etiology of disease have served to deter concerted and cooperative research. The meeting brought the problem into sharper focus and re-emphasized its importance. The relationships of these microbes to arthritis and other diseases in humans, the extent of their threat to livestock industry, and the fact that they contaminate tissue cultures used in diagnosis and research are factors that have led to growing concern.

PROVING GROUND FOR RAPID
TULAREMIA DIAGNOSTIC TEST
PROVIDED BY VOLES IN OREGON

During the last year or so, voles (Microtus) in the Klamath Falls, Oregon, area were so numerous that they threatened

the agricultural economy of the district. It was estimated that there were as many as 4,000 voles per acre. Studies of the population showed that in limited areas the voles were dying of tularemia and it was possible to demonstrate the causative agent (Pasteurella tularensis) in animals which were only recently dead. When it was found that the natural waters of the region contained the organism, it became a matter of concern to determine the extent of tularemia among the rodents.

A modification of the Ascoli test was used by Drs. Bell, Jellison, Owen and Larson of the National Institute of Allergy and Infectious Diseases Rocky Mountain Laboratory for this purpose. Tissue of animals are ground, suspended in salt solution, and boiled. The fluid is clarified and used in precipitin tests. In order to make the method safe for ordinary laboratory processes, the tissues are heated before being ground. The Hamilton, Montana, investigators report in the Journal of Wildlife Management that there was a significant correlation between the results obtained with this test and those obtained by more laborious and dangerous methods;

consequently identification of tularemia as a cause of death of voles was made primarily by the modified Ascoli test.

As the population of voles in Oregon increased, tularemia continued to be a major cause of death in rodents, and the responsible organism was isolated from natural waters repeatedly. Fortunately, the strains were only of moderate virulence and only a few cases of tularemia were noted in human beings. In Russia, where virulent strains are found under similar conditions, hundreds of human cases have been reported.

Application of Ascoli tests to animals dying in the field serves to identify such diseases as plague and tularemia and to indicate the magnitude of potential source of infection for man and domestic animals. The procedure has also been used by these investigators for the diagnosis of tularemia and plague in man.

BACTERIOPHAGE MAY TRANSFORM
DRUG-SENSITIVE ORGANISM
TO RESISTANT STAPHYLOCOCCUS

In a study of gene transfer between staphylococcal cells via the typing phages which parasitize these cells,

Dr. M. L. Morse, National Institute of Allergy and Infectious Diseases grantee at the University of Colorado Medical Center, Denver, reports that about one phage particle in $10^7 - 10^8$ transfers to its bacterial host the genes for resistance to streptomycin and novobiocin.

Among several different staphylococcal typing phage preparations studied, the National Collection of Type Culture, London 8406 lysates were found to transfer genes for resistance when parasitizing the 8511 staphylococcus strain.

The transduction studies reported in the Proceedings of the National Academy of Sciences recently were performed by mixing phage (cells are induced to produce quantities of these viruses by ultraviolet radiation) with phage-sensitive cells at multiplicities of one phage or less per cell. The mixture was then allowed to incubate at 37°C for 5-27 minutes, during which time 95 percent of the added phage adsorbed to the cells.

Phage grown in streptomycin-resistant cultures of staphylococci was transferred to bacterial cultures known to be sensitive to the antibiotic. Transduction of the genes for resistance to streptomycin and novobiocin was observed only when resistant cultures were the source of the phage. The number of drug-resistant clones produced by this transduction is proportional to the amount of phage employed. In the case of novobiocin, the newly acquired resistance became immediately apparent. With streptomycin there was a 4 to 6 hour lag.

The Denver investigator comments that the limitations on the number of genetic markers found transferred by the phage method is owing to the lack of genetic factors for which selection can be exercised. Since the nutrition of the strains employed is nearly defined, it is expected that requirements for amino acids and vitamins, and the utilization of carbohydrates will become available shortly for further study of this transduction system.

SHIGELLOSIS DEMONSTRATED TO CAUSE SEVERE DYSENTERY AND DEATH IN FAMILY OUTBREAK

Local newspapers in western Montana carried stories concerning a "mysterious malady" responsible for the death of two children in a family of ten. Only the father escaped infection, while the mother and eight children ranging from 1 to 10 years of age developed typical symptoms of shigellosis. One child died before and the other after hospitalization. The patients were treated with various antibiotics.

At the time of examination by investigators from the National Institute of Allergy and Infectious Diseases' Rocky Mountain Laboratory, it was not possible to demonstrate organisms in the stools or antibodies in the blood. After antibiotic therapy had been discontinued and a suitable time had elapsed, it was possible to demonstrate Shigella sonnei in the stools of four individuals and antibodies against this organism in blood specimens of all survivors. The father had neither organisms nor antibodies.

This study demonstrates that shigellosis may be a severe and fatal disease, and may present problems in diagnosis, especially if antibiotic therapy is started before stool specimens are taken for bacteriological examination. It had been the general opinion that this organism causes a summer illness and is not found in the northwestern United States, but this study reveals that S. sonnei may be present in this area during the early spring season. Results of the investigation have been reported by Mary L. Casey and Bettie Smith of RML in the Rocky Mountain Medical Journal.

CELL BIOLOGY

RESEARCH PROGRESS ON AMINO ACID METABOLISM IN MAMMALIAN CELL CULTURES SUMMARIZED

Dr. Harry Eagle, Chief, NIAID Laboratory of Cell Biology, summarizes progress in research on the metabolism of amino acids in mammalian cell cultures in Science.

A number of cell lines from normal and malignant tissues have now been serially propagated. Cells and medium can be

separately analyzed, balance experiments set up, metabolic processes examined, and corresponding enzymatic activities explored in cell-free extracts.

In discussing recent findings on the metabolic roles of various components (amino acids, carbohydrate, salts, vitamins, serum protein) of a minimum essential medium for cultivation of mammalian cells, Dr. Eagle notes several refinements in the medium: for example, the concentrations of most of the amino acids are greater than those originally recommended in 1955 and 1957 publications, the relative amounts conforming more closely to the protein composition of cultured human cells. This permits the cultures to be kept for somewhat longer periods without refeeding.

While eight amino acids suffice for nitrogen balance in man (Rose), every cell culture so far examined has required at least thirteen for survival and growth. Several possible explanations have been advanced for this. Experiments with fresh first culture passage monkey kidney cells have shown that this additional requirement is not due to loss of biosynthetic mechanisms in the course of prolonged growth; but Dr. Eagle points out that these experiments do not exclude the possibility that the necessary enzymes are lost from the cells within the first few hours after their removal from the animal.

Cells in culture have a rapid growth characteristic which might outpace capacity for biosynthesis of the additionally needed nutrients. Experiments using C^{14} labeled glutamic acid and N^{15} ammonia have shown that although glutamic acid at physiological levels usually does not substitute for glutamine in these cell cultures, cells do have a limited capacity to make glutamine from these precursors. As an exception to the general rule, monkey kidney cells in primary passage can use glutamic acid or glutamine interchangeably, and in these cultures the minor biosynthesis of glutamic acid from glucose permits prolonged survival and occasionally limited growth, in a medium lacking both glutamine and glutamic acid. A similar limited biosynthesis by human cells could account for the fact that glutamine is not necessary for nitrogen balance in short-term feeding experiments in man. Mention is made in the Science paper of unpublished work showing that certain cells can form an enzyme which permits them to grow at low levels of glutamic acid for long periods and perhaps indefinitely. This observation, growing out of research by De Mars, may be the first case of enzyme induction in serially propagated mammalian cell cultures.

Along with glutamine, arginine, histidine and tyrosine, the amino acid cyst(e)ine is an additional need of cultured cells.

The report in Science draws data from a paper in preparation (Eagle, Oyama, Piez, Fleischman) in demonstrating that four general pathways are used by cultured human cells for the provision of cyst(e)ine. For example, the serum protein additive is a partial source. It remains to be seen whether cannibalization of dispensable cell proteins is another partial source of cyst(e)ine.

The biosynthesis of the "nonessential" amino acids is also under investigation by the NIAID Laboratory of Cell Biology. The specific source of the α -amino nitrogen of the eight nutritionally nonessential amino acids is one of the facets now under study. In one group of experiments, small cell populations have been found to have special nutritional requirements. For example, preliminary results indicate that the major factor involved in this anomalous requirement for a nonessential amino acid, serine, by small cell populations is the loss of serine from the cell pool into the medium at a rate which exceeds the biosynthetic capacity of the cell.

Dr. Eagle further discusses the amino acid pool which has been found to be a significant component of all cultured human cells thus far examined. The composition of the pool in culture is essentially similar to that in animal tissues. Glutathione, taurine, glutamine, ammonia, and glutamic acid are present in largest amounts and together constitute approximately 60 percent of the total pool amino acids. It has been possible to determine for a number of specific amino acids the average intracellular concentration necessary for the initiation of protein synthesis and cell growth.

An understanding of metabolism of essential amino acids and of protein turnover in the cultured cell, outlined in the Science report, has permitted studies of the role of amino acids in the biosynthesis of poliovirus. Dr. Eagle relates data from several papers in press or recently published in describing progress in this area. An early study left open the question as to whether the viral protein was formed from the amino acid pool of the cell, whether the cells utilized their own protein for viral synthesis, or whether these two possibilities were the same, in the sense that cell protein turnover could supply the necessary free amino acids. Recent observations indicate that the synthesis of poliovirus by the HeLa cell requires the presence of a full complement of amino acids in the pool. In contrast to the results obtained in large cell populations, when a relatively small number of cells were placed in a large volume of medium containing only glucose, glutamine, and salts, the amount of virus formed per cell was strikingly reduced. In this situation there is a rapid loss of amino acids from the cell pool; and the capacity of the cell to form virus was restored by the addition of a full complement of amino acids

to the medium. It is clear that the optimal poliovirus synthesis by the HeLa cell requires the presence of free amino acids in the pool, and it is a reasonable presumption, borne out by recent experiments with labeled amino acids and purified virus (Darnell and Levintow) that these amino acids are used by the synthesis of viral protein.

Although the basal medium has been employed successfully for the cultivation of cells directly from the animal host, there is no information as to the proportion of cells which grow out, or as to the additional growth factors which would be required for maximally efficient cloning. Until such cloning can be achieved with essentially 100 percent efficiency, the identification of cell cultures, as for example, "liver," "lung," or "bone marrow" is suspect. Dr. Eagle further notes that specialized organ functions are conspicuously absent in almost all serially propagated dispersed cell cultures. It remains to be determined whether this reflects incorrect identification of the cells, an irreversible loss of their biosynthetic capacities, absence from the environment of necessary precursors or cofactors, or the fact that cellular organization and interaction are essential to those specialized functions.

AUREOMYCIN INHIBITS ENERGY
TRANSPORT MECHANISM OF
BACTERIAL CELL

Aureomycin, one of the so-called broad spectrum antibiotics, apparently acts by inhibiting the bacterial

cell's mechanism for producing and using energy. This observation by Dr. Arthur K. Saz and Mrs. L. Marina Martinez of the National Institute of Allergy and Infectious Diseases represents the first clear demonstration that a clinically-useful antibiotic can inhibit a bacterial, cell-free enzyme complex. The investigators reported their findings at the 1959 meetings of the Society of American Bacteriologists in St. Louis. They also found evidence that may help explain how antibiotic-resistant forms of bacteria emerge.

The researchers employed ultrasound to crack the cell walls of Escherichia coli bacteria. From the exposed cytoplasm within the cells they then obtained, by various biochemical techniques, highly purified, soluble constituents containing the enzymes concerned with energy formation and utilization.

In test tube studies, the electron transport system (energy forming system) from sensitive E. coli was inhibited by Aureomycin. However, if the enzyme system came from a resistant strain of the bacteria, it was not thus inhibited.

The researchers found that the locus of Aureomycin inhibition was a portion of the system which has Vitamin B₂ as an essential component, but attempts to identify the precise mechanism of sensitivity have not been successful thus far. When both antibiotic-sensitive and antibiotic-resistant enzymes are further purified, their energy processing activity diminishes to a vanishing point. Stimulation of this activity up to 2000 percent is achieved by adding small amounts of human or bovine blood serum albumin--but now both types of enzymes prove to be sensitive to Aureomycin. The vitally important factor, whatever it may be, which causes a difference in reaction to antibiotics, is lost during purification of the enzyme.

The investigators point out that their work is significant in demonstrating that resistance may develop in a most subtle fashion. Aureomycin-sensitive and resistant bacteria for the most part are similar, possessing almost interchangeable energizing enzymes. The resistant form apparently is resistant merely because it has synthesized a slightly altered enzyme complex.

Little is known of the modes of action of antibiotics, still less about the biochemical mechanisms involved in the emergence of resistant forms of bacteria. Elucidation of these processes will, at the applied level, provide a rational approach to the therapy of infectious diseases. On a basic level, an understanding of the main energy-yielding processes of cells (electron transport) and the significance of both stimulation and inhibition of these enzymes is of great theoretical significance, since the cell is a basic unit of all life.

VARIANT CELLS CAN BE SELECTED FROM NORMAL POPULATIONS BY NUTRITIONAL MANIPULATION

Rarely occurring auxotrophic cells, whose auxiliary nutritional need is for glutamine, have been obtained from large populations of cells in test tube cultures by a method which a cell biologist of the National Institute of Allergy and Infectious Diseases described at the 1959 meetings of the Federation of American Societies for Experimental Biology.

Dr. Robert I. De Mars reported that genetic studies of human cells may benefit from this technique for selecting out nutritional mutant cells. Their extra need for a growth factor provides both the means for isolating them from nutritionally normal cells and the marker by which they can be traced through successive generations.

In this method, populations of cells in culture are artificially starved for thymine. Initially, it is not apparent which are nutritionally normal and which are not. Thymine starvation is induced by aminopterin or by 5-fluorodeoxyuridine, which prevent the cells from synthesizing thymine, one of the nutritional elements necessary to cell proliferation. This thymine starvation leads to the death of nutritionally normal cells.

However, prior to this, the auxotrophic mutants among the cells have reached a state of virtual suspended animation because of their unsatisfied need for an extra nutritional factor--glutamine, for example. Subsequent thymine starvation fails to kill these sleeping cells. They can be reactivated by discontinuing the artificial starvation of thymine and adding the needed glutamine nutrient.

Ultimately, according to Dr. De Mars, one might hope to employ these genetic mutants in attempts at developing linkage maps such as have been obtained for fruit flies, maize, and micro-organisms--the materials of classical genetics. The variant cells should also prove useful in studying the particular types of alterations in genetic materials that occur in human cells and factors which influence the frequency of occurrence.

SERUM ISOANTIGENS PROVIDE NEW CHEMICAL TOOL FOR GENETIC STUDIES IN MAMMALS

Serum proteins of one species produce (hetero) antibodies when injected into another species. Previous studies by

Dr. Sheldon Dray and Miss Glendowlyn O. Young of NIAID's Laboratory of Immunology have shown that in domestic rabbits serum proteins of one individual will produce (iso) antibodies when injected into other individuals of that species. The production of such isoantibodies was until recently considered impossible.

After previously establishing the isoantigenicity of alpha, beta and gamma globulins in rabbits, the investigators turned their attention to determining the number and incidence of different gamma globulin isoantigens in normal rabbits. Dr. Dray reported these new findings at the 1959 meeting of the Biophysical Society in Pittsburgh.

The antibodies produced are capable of precipitating the specific normal rabbit gamma globulin which was injected. These isoprecipitins were used for the agar gel immunochemical analysis of the sera from 500 normal domestic rabbits of several breeds. This analysis showed that individual rabbits contained one or the other or both of two different gamma globulin isoantigens in their sera but never lacked both of them. Of the 500 rabbits tested, 24 had one type of gamma

globulin, 379 had a second type, and 97 had both types in their sera. The investigators have not yet been able to find a third ~~gamma~~ globulin isoantigen.

The most familiar isoantigens are those which are the basis of the blood groups of man, namely the A, B, and Rh isoantigens present in the red blood cells. Their significance in blood transfusions, in erythroblastosis fetalis (a disease of the newborn), and in forensic medicine (paternity suits, etc.) is well known. These and other known isoantigens are carbohydrates. In contrast, the gamma globulin fractions now being studied by Dray and Young are the first well characterized protein isoantigens; indeed, these protein isoantigens are the only ones known thus far which occur naturally in serum, in contrast to A, B, and Rh isoantigens which are attached to cells.

Serum isoantigens provide a new chemical tool for genetic studies in mammals and for the zoological differentiation of species and may be particularly useful in approaching some of the theoretical problems of immunology, such as the nature of antigen and antibody sites, the question of antigenicity of antibodies, and the mechanisms of antibody formation. In addition, these proteins may be implicated in some of the unexplained transfusion reactions in man and may be useful in the investigation of immune tolerance, serving also, perhaps, as an experimental model for research on the moot question of tolerance to Rh antigen.

SYNTHETIC POLYPEPTIDES OF LYSINE INHIBIT SOME ASCITES CARCINOMA IN MICE

After laboratory demonstrations by a number of investigators of the antimicrobial action of polylysine and other synthetic polypeptides, these compounds as well as commercial protamine sulfate and synthetic polypeptides of 1. glutamic acid, 2. valine and lysine, and 3. leucine and lysine have been tested against Ehrlich ascites carcinoma in Swiss mice. Reported in the Proceedings of the Society for Experimental Biology and Medicine, the research was conducted at the University of Wisconsin by T. Richardson (Predoctoral Fellow of the National Cancer Institute), J. Hodgett, A. Lindner, and M. A. Stahmann, with grant support from the National Institute of Allergy and Infectious Diseases and the Wisconsin Alumni Research Foundation.

Young adult mice weighing 20 to 25 grams were each transplanted intraperitoneally with 5 to 10 million tumor cells. Treatment was begun 24 hours after tumor transplant and generally consisted of 2 intraperitoneal injections of polypeptide solution daily for 6 days. Only the synthetic polypeptides of lysine

inhibited ascites tumor development. In tests against tetraploid Ehrlich ascites carcinoma, up to 27 percent of the mice treated with polylysine survived for 100 days apparently free from tumor. Mice that died after 3 to 4 weeks usually did so from metastatic solid tumors or solid tumors at site of implant. Polylysine also had a marked inhibitory activity against the diploid strain of Ehrlich ascites carcinoma and TA3 ascites carcinoma in Swiss and BAF1 mice, respectively. Survival time of some BAF1 mice increased to 86 days, while 10 percent of Swiss mice survived 100 days with no sign of tumor. On cytological analysis, mitotic arrest at prophase was found. The nucleic acid and protein content/cell remained unchanged. The investigators hypothesize that antitumor action of lysine polypeptides requires intimate contact with the tumor cells and that refractoriness of solid tumors might mean insufficient drug had reached some cells. High affinity of polylysine for red blood cells and tissue proteins could limit systemic availability of the compound.

The researchers conclude that polylysine has marked cytotoxic effect against ascites tumor cells within the peritoneum of the mice.

PARASITIC DISEASES

CHLOROQUINE PLUS OTHER DRUGS	Chloroquine in combination
MAY DOUBLE PROTECTION	with certain other drugs may
AGAINST MALARIA	result in more than doubled
	protection against malaria,

according to experiments reported by Drs. L. E. Gaudette and G. Robert Coatney during the Eighth Annual meeting of the American Society of Tropical Medicine and Hygiene.

After treatment with 100 micrograms of chloroquine, mice experimentally infected with Plasmodium berghei were free of infection for 4 days. With the same dosage of the drug, plus equal amounts of an auxiliary "extender" drug, protection against the disease increased to 8 days. Isoniazid, pyrimethamine, and two experimental drugs (Eli Lilly 18947; Smith, Kline and French 525-A) have been found to extend chloroquine activity, and other inhibitor drugs are also being tested.

If the results reported can be applied to man, chloroquine combined with a drug prolonging its action could be administered at intervals of 3 to 4 weeks and possibly several months. This improvement from present weekly dosages would be of tremendous benefit in areas of the world where suppressive use of chloroquine against malaria is indicated because of the impracticality or ineffectiveness of insecticide spraying of dwellings for malaria eradication.

EXPERIMENTAL VACCINES PRODUCE EVIDENCE OF IMMUNITY DIFFERENCE IN LIVE VS. KILLED AGENTS Evidence pertinent to the complex question of live vs. killed virus vaccines is provided in the work of Dr. Leon Jacobs and Marjorie L. Melton of the Laboratory of Parasitic Diseases, NIAID.

Working with the parasite, Toxoplasma gondii, the investigators employed various routes of inoculation and dosages of their experimental vaccines. Results with a live but relatively mild strain of the organism not only enabled guinea pigs to survive later challenge with a virulent strain but prevented these invaders from establishing themselves in guinea pig tissues. With vaccines prepared by killing the parasites with heat or with chemicals such as phenol or formalin, the guinea pigs did not die but the T. gondii parasites introduced at challenge were able to proliferate to some extent.

When live T. gondii were used in a vaccine, the "quality" of immunity seemed higher. Even when challenge was delayed to a point where antibodies produced by vaccination had diminished to levels approximating those obtained with killed vaccine, the extent of proliferation of the parasite in tissue was less with the live-agent vaccine.

T. gondii is the causative agent in toxoplasmosis, a sporadic disease to which there is high naturally-acquired immunity in the general population in the United States. For that reason, the research described is not directed toward production of vaccine but toward understanding of the general mechanisms involved in immunity to intra-cellular parasites. Although this protozoan organism is biologically different from a virus, it is similar in that it is an intra-cellular organism.

INTESTINAL PARASITISM Research on intestinal
AMONG AGRICULTURAL parasitism in a group of
LABORERS STUDIED agricultural laborers was
reported by Miss Elizabeth

Guinn, based on a study in the Clewiston, Florida area. One hundred and forty stool specimens from British West Indian workers, aged 21 to 48 years, were tested. Miss Guinn and her co-investigators, Drs. Henry K. Beye and Charles M. Brooks of the Laboratory of Clinical Investigation, found multiple parasites in 66 percent of the specimens.

Hookworm was the most prevalent, occurring in 54 percent of the stools. Workers residing in the United States for less than 1 year had a higher incidence of hookworm than those living here more than a year, 68 and 41 percent, respectively.

Eight other parasites (Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura, Entamoeba histolytica, Giardia lamblia, Entamoeba coli, Endolimax nana, Iodamoeba butschlii), all commonly found in this country, were counted in significant numbers.

There are approximately 1 and $\frac{1}{4}$ million migratory agricultural laborers in the United States, half of whom are foreign nationals and half U. S. citizens from the southern states. In addition to the work force itself, populations at potential risk may be individuals and communities closely associated with camp areas and those which might be affected as a result of a breakdown in sanitation. Especially vulnerable to contamination are water supplies and processed foods.

MINUTE DOSAGES OF PRIMAQUINE MAY BREAK MAN-MOSQUITO-MAN CYCLE IN MALARIA

An exceedingly small amount of the drug primaquine, given daily to each malaria-infected person, may be sufficient to

help break the man-mosquito-man cycle of malaria, according to Dr. Martin D. Young, Head of the Epidemiology Section in the Laboratory of Parasite Chemotherapy, Columbia, S. C.

Two or three milligrams or less of primaquine daily, following a weekly dose of about one-half grain, caused an incomplete sexual cycle of malaria parasites in human blood after their ingestion by the biting mosquito. Normally, gametocyte forms of malaria parasites generate sporozoites, some of which enter the salivary gland of the mosquito where they can be injected into man when the insect feeds. This infection in man may lead to recurrent malaria attacks.

The small doses of primaquine which interfered with this cycle were given to patients with Plasmodium vivax and P. falciparum.

This method of malaria control could provide a stop-gap measure to check further spread of the disease in conjunction with drug therapy and residual spraying with mosquito-killing compounds like DDT. A major obstacle in using primaquine in this way is the difficulty of administering it routinely to each of the estimated quarter-billion of the world's population who are afflicted with the disease.

EFFECT OF DRUGS AGAINST EXPERIMENTAL SCHISTOSOMIASIS ENHANCED BY GLYCERIN

Dr. George W. Luttermoser of the Laboratory of Parasite Chemotherapy, National Institute of Allergy and

Infectious Diseases, reports that the administration of tartar emetic or of stibophen solution in glycerin increases the effectiveness of the drugs against experimental infections of Schistosoma mansoni in mice. The results of his investigations

appear in the Journal of Parasitology.

White mice exposed to about 250 S. mansoni cercariae each were held for at least 35 days before treatment was started. By this time the parasites were mature. Only animals free of gross signs of illness and differing no more than 1 or 2 grams in body weight were used. Groups of usually 30 or more mice which had been infected at the same time were divided into 3 lots. The first lot was maintained as the untreated control, the second received stibophen intraperitoneally or tartar emetic orally in physiological saline, while the third lot was given the same regimen of the drugs in glycerin.

Drugs were given on the basis of body weight and treatment continued daily for 5 consecutive days. Appraisal of treatment was made the third week after treatment. Live parasites remaining in each mouse were perfused out and counted. A comparison between the average number of living schistosomes in the treated and untreated groups gave the approximate percentage reduction in infection resulting from treatment.

In early trials the drugs were consistently more effective in glycerin. In 4 tests there was a 51 percent reduction in infection following the regimen of tartar emetic in saline, but a 74 percent reduction from the tartar emetic in glycerin. The activity of stibophen was increased considerably by glycerin: a 32 percent reduction with saline, and a 79 percent in glycerin. Further trials showed similar enhancement of stibophen in 8 or 9 tests; and of tartar emetic in 3 out of 4 tests.

In considering the various means by which glycerin might enhance the schistosomacidal activity of stibophen and tartar emetic, the investigator pointed out that the latter drug is more stable in glycerin solution, suggesting that in vivo the adjuvant may maintain the blood level of the drug for a longer period of time, and thus adversely affect the schistosome parasites in the blood.

Results of his study, concludes Dr. Luttermoser, suggest a further testing of these combinations in larger animals. The effect of glycerin on the activity of additional heavy metal compounds against this infection and others might profitably be investigated.

EXOERYTHROCYTIC PARASITES
DEMONSTRATED TO BE OF
MALARIAL ORIGIN

The studies of Dr. Don E.
Eyles, Head of the Cytology
Section of NIAID's Laboratory
of Parasite Chemotherapy,

bolster the validity of currently accepted findings on the

exoerythrocytic stages of malaria in the liver of primates. Dr. Eyles, working in Memphis, Tennessee, injected monkeys with large numbers of uninfected salivary glands of *Anopheles* mosquitoes. No exoerythrocytic parasites or any structures which could be confused with them were found. Subsequently, smaller numbers of infected glands were injected into the same monkeys and produced many typical EE parasites.

Convincing evidence that the parasites are of malarial origin was provided by chemotherapeutic experiments. Treatment with pyrimethamine and primaquine prior to inoculation prevented the appearance of EE parasites in the liver. When the infection was treated after it was established, the parasites were damaged and died. Histologically, this could be followed clearly.

Dr. Eyles believes that these experiments indicate beyond any reasonable doubt that the structures seen are in fact pre-erythrocytic malaria parasites and not artefacts which could be confused with EE parasites.

HYDATID DISEASE IN HUMANS
CONSIDERED HEALTH HAZARD
IN SOUTHERN UNITED STATES

Hydatid disease or echinococcosis, caused by infection with the dog tapeworm, Echinococcus granulosus,

constitutes a recognizable health hazard in Mississippi, Tennessee, Louisiana and other southern states. This conclusion by NIAID grantees Drs. Thomas J. Brooks, Jr., Watts R. Webb, and Kenneth M. Heard of the University of Mississippi School of Medicine, is documented by a significant though small series of human cases reported in the AMA Archives of Internal Medicine. Three of the seven patients had never been outside Mississippi; a fourth was presumed to have lived there always. Two others were native-born Mississippians who resided in Tennessee; the last case was reported from Louisiana.

The authors point out that some contradictory or erroneous statements have appeared in the voluminous recent literature on hydatid disease. For example, it has been categorically stated by some that echinococcal disease is always contracted outside the continental limits of the United States. This seems to be refuted by the evidence in Dr. Brooks' report.

Hydatid disease is highly endemic in agricultural areas of Africa, parts of South America, and in Tasmania, and is considered a scourge in Australia and New Zealand. Frequent infection in humans has been reported from Europe, Siberia, Turkistan, Mongolia, North China, Japan, the Philippines, Syria, Lebanon, Palestine and Arabia.

In this country the peril to health is of special importance among farmers and those in allied occupations, where there is close contact between man, sheep, pigs, cattle and dogs.

The ova of the tapeworm, swallowed by man or animals, penetrate the intestinal wall and are swept into the portal circulation. Echinococcal cysts have been reported in virtually every organ and tissue in the human host, with the liver and the lungs being the two most common sites.

Attempts to find important definitive hosts of E. granulosus have been largely unsuccessful. Only a few instances of natural occurrence in dogs have been recorded in the United States. The authors believe that the numbers of infected animals must be considerably greater than reports indicate. This smallest parasitic cestode imbeds itself so deeply into the mucous membrane of the small intestine that it is frequently overlooked. Even in animals infected experimentally it is extremely difficult to find.

The investigators caution that this disease will be seen with increasing frequency in the United States unless active control measures are maintained.

MODE OF ACTION OF ANTIMALARIAL DRUGS INVESTIGATED

Consistent results on nucleic acid metabolism were demonstrated for fast-acting antimalarial drugs such as

quinine, chloroquine and quinacrine in tests by Drs. Karl A. Shellenberg and G. Robert Coatney of the Laboratory of Parasite Chemotherapy, NIAID. These clinically effective drugs are part of a series whose mode of action against malaria is being investigated.

Acid soluble, lipid RNA and DNA phosphorus fractions were isolated and their specific activities determined in tests with more than 15 different drugs. At concentrations 10^{-5} M or higher, RNA uptake was inhibited an average of 70 percent and DNA 90 percent by quinine, quinidine, cinchonine, cinchonidine, quinacrine and chloroquine. Other substances which were tested at the same concentrations and proved ineffective were various antibiotics, pentachlorophenol, stilbamidine, ouabain, aminopterin and amethopterin.

EXPERIMENTS SHOW MALARIA PARASITES QUICKLY DEVELOP RESISTANCE TO PYRIMETHAMINE

During various clinical tests of pyrimethamine, an anti-malarial drug, relapsing infections have responded less

well to the second than to the initial treatment. Drs. Martin D. Young and Robert W. Burgess, NIAID Laboratory of Parasite

Chemotherapy, Epidemiology Section, Columbia, S. C. , have conducted experiments designed to determine some of the factors in the occurrence, persistence and transmissibility of resistance.

As reported in the Bulletin of the World Health Organization P. vivax infection (Korean, St. Elizabeth or Chesson strain) was induced in 17 neurosyphilitic patients. Pyrimethamine in single doses of either 25, 50, 100 or 200 mg. was given to test the schizontocidal and sporontocidal effects. The first single-dose treatment of 25 mg. or 100 mg. was given between the 8th and 61st days of parasite patency and gave moderately rapid schizontocidal and very rapid sporontocidal effects. All observed cases relapsed.

The second treatment, usually 3 weeks or longer after cessation of the first and with the same or higher doses, had either a diminished effect or none on the schizogonous and sporogonous cycles of the malaria parasites. Subsequent treatment, even at weekly intervals, had no effect. The resistant quality was undiminished in subsequent infections transmitted by mosquito bites, by the injection of preserved sporozoites, or by transfusion of infected blood. The resistant character appears to be durable, and was still evident 140 days after initial contact with the drug, during which time the parasite strain had passed through a second patient without contact with the drug and then into a third where the challenge was made. This indicates persistence of the characteristic even without frequent exposure to the drug.

The investigators theorize that their experiments indicate how resistance may develop when large single doses of pyrimethamine alone are given at less than monthly intervals to febrile persons having active P. vivax infection.

ORAL DITHIAZANINE
90 PERCENT EFFECTIVE
AGAINST STRONGYLOIDIASIS

Effective therapy for
strongyloidiasis has been
found in the use of a new
oral preparation, dithiaza-

nine (3,3' diethylthiadicyanobenzene iodide--Eli Lilly). Following therapy, there is permanent disappearance of larvae from stools and duodenal aspirate. This is the first drug in medical history consistently to give these results.

Therapeutic trials on 32 patients gave results that were 90 percent effective, according to a report by Drs. J. P. Muhleisen, VA Hospital, and J. C. Swartzwelder, Louisiana State University School of Medicine, New Orleans. This

investigation is reported in the American Journal of Gastroenterology and was partially supported by a grant of the National Institute of Allergy and Infectious Diseases.

An estimated 35 million of the world's population have strongyloidiasis, with 400,000 cases in this country, primarily in our southern states. Severe clinical infections may cause excessive weight loss and protracted diarrhea, resulting in invalidism. Common symptoms include pain in the epigastrium, nausea, vomiting, anorexia, diarrhea and fatigue. Urticaria also is relatively prevalent, a manifestation seldom associated with other roundworm infections.

To treat the infection, a dosage of 200 mg. of dithiazanine was given 3 times daily for 5 days. The treatment period was increased to 21 days when it became obvious that a 5-day course was inadequate. The investigators realized that the shorter course would not affect the larvae migrating extraintestinally as a result of internal or external autoinfection and that reinfection of the upper small intestine might occur. When the 21-day course proved adequate, dosage was reduced from 600 to 300 mg. per day, without loss of therapeutic efficiency.

After therapy, diagnostic forms of S. stercoralis were not demonstrable in stool or duodenal drainage in 29 of the 32 patients treated. Only mild, infrequent and evanescent reactions to the drug were noted. In two cases of death unrelated to therapy, it was possible to make a thorough post mortem examination of the upper small intestine. This revealed no parasites, thus providing additional and conclusive evidence of eradication of the infection.

Dithiazanine is a new oral preparation marketed by Eli Lilly and Co. as Delvex or Telmid. Dr. M. D. McCowen and associates of the Lilly Research Laboratories reported success with the drug in treatment of various helminth infections in laboratory animals. Simultaneously NIAID grantee Dr. W. W. Frye of Louisiana State University and his co-workers reported the drug to be effective against human trichuriasis. The currently reported study on the therapy of strongyloidiasis employed dithiazanine provided by Lilly.

FUNGUS DISEASES

SUCCESSFUL SYSTEMIC TREATMENT
OF SUPERFICIAL FUNGAL DISEASE
ACHIEVED WITH GRISEOFULVIN

According to a study published in A.M.A. Archives of Dermatology by Drs. Harvey Blank and Frank J. Roth, Jr.,

NIAID grantees at the University of Miami School of Medicine--
"The systemic treatment of superficial fungus infections in man at last seems a near reality."

Studies by other investigators had demonstrated that blood-borne substances limit invasion by dermatophytes to cutaneous tissues but are not effective in control of dermatophytosis. More recently the successful oral treatment of experimental dermatophytosis with griseofulvin was considered a highly promising achievement.

The scientists began their present study by administering griseofulvin in a "desperate and unique" case of widespread cutaneous granulomas caused by Trichophyton rubrum. On a daily dose of 5.0 gm., the patient responded promptly. Granulomas healed, lesions subsided and somewhat later diseased nails were replaced with normal growth. No adverse clinical or laboratory signs were manifest.

Following this, the drug was administered to a group of 31 patients infected by such organisms as T. rubrum, T. mentagrophytes, T. tonsurans, M. audouinii, M. canis, and E. floccosum.

In most cases the scientists used a daily dosage level of 1.0 gm. In two cases doses of 4.0 and 5.0 gm. per day were given for one month and then lowered. Examination generally revealed negative cultures in two weeks or less and a negative direct KOH preparation in about two weeks. Hair and nail cultures remained positive until the infected material was removed.

No acquired resistance to the drug and no evidence of anti-mitotic effect have been noted. Counts of the number of mitoses were made in the bone marrows of five patients treated for extended periods with excessive doses. Neither the white or red blood cell series revealed change in the karyokinetic index.

In summary, the mycoses caused by various species of dermatophytes show a uniformly favorable response to oral therapy with griseofulvin. Infections of long duration, up to 60 years in the case of one patient infected with T. tonsurans, seem to respond as readily as those present for a few weeks or months. Tinea corporis is usually cleared in one to two weeks; itching usually ceases in three to five days. Tinea pedis is improved in one to two weeks but may require three to four weeks or longer to clear. Tinea capitis improves in two to three weeks but may require more prolonged treatment. Onychomycosis requires three to four months to clear, but new normal nail growth is seen earlier. The treatment duration will probably vary, apparently depending on the time required for normal replacement of the infected tissues. Likelihood of relapse or recurrence of infection is as yet not known.

Griseofulvin is an antibiotic isolated from Penicillium griseofulvin by Oxford, Raistrick, and Simonet in 1939. The chemical structure of the substance was established and its activity against a number of fungi reported in a series of publications by P. W. Brian, Jr., F. Grove, T. S. Work and others.

Griseofulvin is manufactured by Schering Laboratories under the proprietary name Fulvicin and by McNeill Laboratories under the name Grifulvin.

CRYPTOCOCCOSIS SUSPECTED
IN PNEUMONITIS ASSOCIATED
WITH CONTACT WITH PIGEONS

Isolation of virulent strains
of the fungus, Cryptococcus
neoformans from 63 of 91
specimens of pigeon droppings

was reported at the IX International Botanical Congress in Montreal, Canada, by Dr. Chester W. Emmons, mycologist of the National Institute of Allergy and Infectious Diseases. Dr. Emmons is continuing his investigations of the prevalence of this fungus in the environment in order to estimate the exposure potential for humans. He also questions certain retrospective diagnoses in outbreaks of pneumonitis; for example, an epidemic of this lung disease in Plattsburg, N. Y. (White and Hill, 1950) among 23 men who tore down a school building. The tower was filled with pigeon droppings to a height of four feet. Dr. Emmons believes it probable that C. neoformans, rather than the histoplasmosis fungus as surmised, was the pathogen.

The recent studies in the Washington, D. C., area provide evidence of how frequently pigeon droppings harbor C. neoformans. The fungus was isolated from pigeon droppings in 14 of 15 specimens from barns and warehouses in one series, and from 14 of 25 in another series; it was isolated from 7 of 10 specimens from an old schoolhouse now used as an office building; from 7 of 7 originating in the cupola on a school building; and from 17 of 18 collected from window ledges of federal and municipal office buildings. None of 7 specimens from a city park yielded the fungus, but this was not a sufficient sample for conclusive evaluation.

Emmons comments, ". . . assuming that cells of the fungus must be inhaled frequently, one can only speculate on the reasons for the infrequent recognition of pulmonary cryptococcosis in man. The most probable explanation is that primary pulmonary lesions occur as they do in histoplasmosis, that these lesions heal spontaneously due to innate resistance of the individual, or to immunity acquired during the slow evolution of the pulmonary lesion and that the fungus reaches the central nervous system in only a small percentage of cases."

Cryptococcal meningitis, unless adequately treated, is almost invariably fatal.

STEROIDS AND ANTIBIOTICS MAY
PREDISPOSE CHRONICALLY ILL TO
AIRBORNE FUNGAL INFECTION

Results of an animal study by
Drs. Herschel Sidransky and
Lorraine Friedman of Tulane
University School of Medicine
suggest that chronically ill patients under prolonged treatment with cortisone and broad-spectrum antibiotic agents may become extremely susceptible to pulmonary aspergillosis.

Observing the growing number of secondary fungal infections of the lung among the routine necropsies performed at New Orleans Charity Hospital and aware that most of these patients had received cortisone and antibiotic agents, the authors, supported by a grant from the National Institute of Allergy and Infectious Diseases, initiated an inquiry into a possible causal relationship. The results of their inquiry appeared in the American Journal of Pathology.

The authors injected mice with cortisone and penicillin 2 days before exposing them to clouds of Aspergillus flavus spores. Another antibiotic, tetracycline, was added to their drinking water. Two control groups were investigated simultaneously, one subjected to spores but no cortisone or antibiotic agents, the other to the reverse.

To determine whether cortisone and antibiotic agents given separately and in combination influenced the susceptibility of mice to lethal pulmonary aspergillus infection, the authors performed two experiments. In each they divided mice into four groups and exposed them to high concentrations of spores. One group received cortisone and antibiotic agents, a second received cortisone alone, a third received antibiotic drugs, and a fourth received no supplementary treatment.

Deaths occurred only among mice receiving cortisone alone or in combination with the antibiotics and of these, from 67 to 88 percent died by the seventh day. In the case of a similar group of mice receiving a low concentration of spores, the mortality figure was reduced to 30 percent.

At the time of sacrifice (3 weeks after inhaling spores), the lungs from the control animals were normal in appearance but experimental mice which died following exposure exhibited pulmonary lesions resembling those seen in human cases and consisted of bronchopneumonia accompanied by hyphal invasion of blood vessels.

In another control experiment in which mice were exposed to massive clouds of heat-killed spores, no animals died, either in the group treated with cortisone and antibiotic agents or in the untreated group.

In summary the investigators found that normal mice may inhale large numbers of spores of Aspergillus flavus and suffer only a mild self-limiting bronchitis and pneumonitis. However, pretreatment with cortisone and antibiotic drugs rendered mice highly susceptible to fatal pulmonary aspergillosis, although cortisone rather than the antibiotics seemed to be chiefly responsible for the enhanced susceptibility. The investigators suggest that patients treated in similar fashion may likewise become susceptible to infection with air-borne aspergilli.

RE-EVALUATION OF PUBLIC HEALTH PREVENTION AND CONTROL OF ORAL THRUSH IN NEWBORN DESIRABLE Dr. Philip J. Kozinn and associates advise that a re-evaluation of public health measures for the prevention and spread of oral thrush in nurseries appears desirable. Their report is the result of a series of investigations at Maimonides Hospital, Brooklyn, New York, supported by a grant from the National Institute of Allergy and Infectious Diseases.

Candidiasis, usually a harmless fungus infection in the nursery, may sometimes become systemic and fatal; its most common clinical manifestations are oral thrush and cutaneous lesions.

In the past, certain conditions were generally considered to predispose newborn infants to oral candidiasis. They included: prematurity, low birth weight, unclean nipples and bottles, and antibiotic therapy. However, in a series of 87 premature infants, 3.5 percent developed the disease versus 3.1 percent in a series of 2,175 full-term infants. Of 158 breast-fed infants 4.4 percent developed oral thrush versus 3.4 percent out of 2,017 who were bottle fed. Of the infants who received antibiotic therapy, 2.7 percent developed the disease. The investigators concluded that the infants who developed thrush did not differ significantly from the normal controls with regard to these factors.

While Candida albicans may occur as a harmless saprophyte on the mucous membranes of adults, its presence in the newborn infant is almost invariably associated with clinical manifestations. For some as yet unknown reason, newborn infants have little ability to resist infection with Candida albicans, the oral mucosa being particularly susceptible to colonization by

the organism. The investigators feel it is this intrinsic lack of immunity rather than the factors listed above which predispose young infants to candidiasis. Apparently this defect rectifies itself spontaneously as the infant grows older.

The investigators deny the view still expressed in many textbooks that candidiasis is an airborne disease, pointing out that neither they themselves nor any previous investigator has been able to isolate Candida albicans from any sample of air or soil.

Dr. Kozinn, however, did confirm the possibility that maternal vaginal candidiasis may be a major source of neonatal infection. In one group of 75 mothers, 20 harbored Candida albicans the first day after delivery and 50 percent of their infants subsequently developed the disease. None of the infants born to the 55 mothers with negative mycologic findings developed candidiasis.

Isolation of infants once they develop clinical manifestations is required by the sanitary code in many municipalities. The investigators note, however, that airborne infection has been discredited and potential sufferers appear to harbor Candida albicans from the time of birth and are a source of contagion. The investigators feel that spread of the disease may be prevented effectively if the infants are segregated on the side of the general nursery and handled with strict care.

LARGEST EPIDEMIC OF HUMAN RINGWORM STUDIED IN FARM AREA

What apparently represents the largest single epidemic of human ringworm caused by Trichophyton verrucosum is

reported in the New England Journal of Medicine by Dr. John S. Strauss of the Boston University School of Medicine under a grant from the National Institute of Allergy and Infectious Diseases. The epidemic followed from contact with a single herd of cattle at the Walter E. Fernald State School Farm Colony of Templeton, Massachusetts.

In total, the scientist studied 16 cases of inflammatory ringworm among mentally defective boys, all of whose lesions were on exposed surfaces of the body. Of this number, 6 yielded positive cultures, 5 of them being obtained from 8 relatively non-inflammatory, plaquelike lesions and only 1 from the highly inflammatory lesions. At 37° C. all of these showed the typical white, waxy, folded colonies of the organism, and hairs taken from a subject with scalp lesion and stained revealed the large-spore formation so characteristic of this species.

The farm colony of 300 mentally defective boys is described by the investigator as being divided into four separate groups of unequal size with separate living quarters and eating facilities and with relatively little contact between them. All of the 16 cases studied by Dr. Strauss appeared in one group of approximately 50 boys employed in the care of the dairy herd. Of these only 1 had not come in contact with the herd.

The herd of approximately 160 head was divided into two groups of equal size. Early in 1958 an explosive epidemic of ringworm, involving 80 percent of the animals, was seen in one barn and 2 months later lesions developed in 60 percent of the animals in a second barn. Nearly all of the human cases occurred at this time. Among the infected cattle the doctor found only a few active lesions described as scaly, circumscribed patches varying in size up to several inches.

Although one boy had no direct contact with the infected cows, previous studies supply ample evidence for an indirect means of spread. Positive cultures have been obtained by other investigators from scratching posts and from soil around barnyards, for example. Furthermore, the organism has been demonstrated to remain viable for over 4 years in a dry state.

Commenting on the cultural characteristics of this organism, the investigator observes that its growth is relatively slow and overgrowth with contaminants is common, necessitating agents added to the medium to suppress contaminant molds and bacterial growth. Furthermore, the organism does not grow on ordinary medium and practically all strains require added thiamine; many also requiring inositol. Finally, optimal growth occurs at 37° C., in contrast to room temperature for other dermatophytes. For these reasons, special precautions must be observed when an infection with this organism is suspected. Certainly, many were missed in the past because of ignorance of certain of its cultural characteristics.

RICKETTSIAL DISEASES

INFECTED SHEEP IMPLICATED
IN GROWING PROBLEM OF
Q FEVER IN NORTHWEST

The National Institute of
Allergy and Infectious
Diseases Rocky Mountain
Laboratory in Hamilton,

Montana has investigated factors associated with a marked increase in Q fever in Idaho during 1958. The study by Stoenner and associates at RML is reported in the American Journal of Hygiene.

During 1951, 20 cases of Q fever were noted in Idaho. These occurred predominately in males and in persons having contact

with sheep. During the period from 1951 to 1958, 131 persons were found to be infected. Most of the cases occurred in persons aged 20 to 69 years and only 9 percent were in females. The majority of cases occurred during the months of March to June during the lambing season. It was possible to demonstrate that sheep are infected with Q fever organisms but that the infection tended to be limited in time. Surveys of dairy herds conducted in 1951 indicated that less than 1 percent of the herds were infected. An alarming increase in the number of infected dairy herds was detected in 1958, for 17 percent of the herds were found to be infected.

These results indicate that Q fever has become a serious problem in the northwestern United States and that sheep and cattle are widely involved. While the epidemiologic data presently available indicate that human disease is most often contracted from sheep, the recent widespread increase of infection in cattle has built a potential reservoir of infection for man which must be reckoned with in the future.

LIVE Q FEVER ORGANISMS PRESENT IN WATER AND SOIL DURING FULL SPAN OF LAMBING SEASON A cooperative investigation of the extent to which infected soil and water might serve as sources for secondary infective aerosols in the spread of Q fever has revealed that viable rickettsiae were continuously present in the soil up to 150 days, an interval encompassing the entire lambing season. Also, the concentrations of rickettsiae encountered in the soil suggested that secondary (dust) aerosols may lead to infection of man and livestock in the absence of active shedding of rickettsia by infected animal hosts.

A study of six sheep ranches in Solano County, California, was conducted by investigators from the California State Department of Public Health and the Communicable Disease Center, PHS, under grants from the National Institute of Allergy and Infectious Diseases and the Army's Chemical Corps Biological Laboratories. Hartwell H. Welsh, Edwin H. Lennette, Francis R. Abinanti, John F. Winn, and William Kaplan reported their findings in the American Journal of Hygiene.

Examination was made of 19 water samples and 235 soil samples collected on a weekly basis over a 10-month period on 5 different ranches and for a 15-month period on the sixth ranch. All ranches were located in central California, an endemic and enzootic area of Q fever, and all harbored flocks of sheep with evidence of past or present infection with Coxiella burnetii. All samples were taken from the lambing area starting shortly after the peak of one lambing season and continuing to the beginning of the next season in five cases, and through the subsequent season in the last instance.

Supernatant fluids from soil sample preparations treated with 400 units per ml of penicillin and fluid samples similarly treated were injected into hamsters used in groups of four per specimen. Each animal received 1 ml of the inoculum intra-abdominally. The inoculated animals were held 6 weeks, then bled. Their sera was examined for the presence of complement-fixing antibody to C. burnetii. Development of the antibody was interpreted as evidence that the inoculum contained the rickettsia.

In summary, the investigators assumed that recoveries of C. burnetii from the air at times of the year other than during the lambing season (when parturition itself has been shown to generate infective aerosols of considerable magnitude and duration) are due to disturbances of contaminated ground surfaces. Disappearance of the rickettsia from the sampling spots occurred after termination of the lambing season, and evidence was obtained that recontamination of the environment occurred during the succeeding lambing season.

NATIVE FAUNA SPOTLIGHTED AS INFECTIOUS SOURCE FOR MAN AND DOMESTIC ANIMALS

During the period from 1954 to 1957 a cooperative project to study the ecology of certain infectious diseases was carried out by NIAID's Rocky Mountain Laboratory and the Army's Dugway Proving Ground. Over 6,000 mammals and 41,000 ectoparasites were examined. A total of 145 isolations of Rickettsia rickettsii was made from 643 pools of various species of ticks. Serological evidence of previous infection was noted in 289 of 2,000 specimens examined. Evidence pointed to jack rabbits and Ord kangaroo rats as significant factors in maintaining a reservoir of spotted fever rickettsia.

Coxiella burnetii, the causative agent of Q fever, was isolated twice from only one species of tick, but evidence of its presence in local animals was relatively abundant during certain periods. Thus, in one period, 30 percent of 75 animals possessed antibodies against C. burnetii. The organism was isolated from a number of specimens of kangaroo rats. It is postulated that C. burnetii has one cycle in nature and another independent one in domestic livestock, but the exact relationship between these two is unknown.

A new species of Brucella, Br. neotomae, was isolated from wood rats and Br. suis was obtained from a jack rabbit. Isolations of plague bacilli and tularemia organisms were also made.

These studies emphasize the importance of local fauna as sources of infection for man and domestic animals. In view of the present rapid spread of Q fever in domestic animals,

consideration must be given to resident animals as sources for transmission of the agent. Previous to these studies, Q fever had been detected in domestic livestock but had not been detected in the resident fauna of the United States. This work by Dr. H. G. Stoenner and associates of the Rocky Mountain Laboratory was published in the American Journal of Tropical Medicine and Hygiene.

EXPERIMENTAL PARALYSIS IN
SMALL ANIMALS TESTS SUSCEPTI-
BILITY TO ROCKY MT. WOOD TICK

A well known cause of the
sometimes fatal tick paralysis
of man and some animals in
parts of Western United States

and Canada are the engorging females of the Rocky Mountain wood tick, Dermacentor andersoni. Even though millions of ticks, including wild-caught adults, have been fed on laboratory animals during spotted fever vaccine studies and various other investigations, only in rare, isolated instances has recognizable paralysis been seen in these hosts.

Upon discovering that hamsters became paralyzed when bitten by adult progeny of British Columbia ticks which had produced paralysis in dogs, Mr. Lyndahl E. Hughes and Dr. Cornelius B. Philip of the Rocky Mountain Laboratory initiated a study to test the susceptibility of various laboratory and native Montana animals to this infection. Wild-caught D. andersoni adult ticks from B. C. and the Bitterroot Valley in western Montana were fed, by means of capsule bandages, on ground squirrels, woodchucks, wood rats, rabbits, cats, dogs, hamsters, guinea pigs, white rats and a rhesus monkey.

Not all ticks fed on the various animal hosts produced paralysis, but the highest percentage of transmission was by those from British Columbia. Positive tests for the disease were obtained in hamsters, guinea pigs, ground squirrels, woodchucks, wood rats, dogs, and the monkey, but ticks completed engorgement on white rats, rabbits, and cats without producing disease.

The signs of illness in the various animals tested did not vary greatly, with the exception of an eye condition observed in almost all hamsters and in two ground squirrels. The eyes in these animals became closed with a copious, conjunctival exudate. The first signs of the infection, the investigators say, are a short period of hyperactivity and then incoordination of locomotion. In a few hours progressive flaccidity develops, followed by inability even to raise the head. In most instances, the disease was fatal to small animals when ticks were about half or even less engorged; however, two hamsters recovered after complete flaccidity while female ticks were still feeding on them. The only gross lesions observed at necropsy are hyperemic congestion of lungs with

some excess pleural fluid and a suggestion of inflammation of small intestine.

British Columbia adult ticks, reared from the previous generation that paralyzed dogs, caused paralysis in 34 of 79 hamsters in 4-8 days after attachment. The disease was fatal in 22 of the animals and 12 recovered--two spontaneously and ten upon removal of the ticks.

The susceptibility of hamsters was illustrated by a series of tests in which these animals, recovering from one paralytic attack, were subjected again to the bite of the B. C. ticks and, following paralysis, made a second recovery after early removal of the ticks.

A color film recording the various stages of paralysis in some of these animals was shown at the 1959 meeting of the American Entomological Society in Salt Lake City.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

ARTHRITIS AND METABOLIC DISEASES

1959

Items of Interest on Program Developments and Research Studies
Conducted and Supported by the National Institute of Arthritis
and Metabolic Diseases

The year 1960 marks the tenth anniversary of the National Institute of Arthritis and Metabolic Diseases. It has been a ten year period of remarkable growth, highlighted by advances in both the basic sciences and in clinical medicine.

In the rheumatic disease field the Institute is now supporting a considerable amount of research on hypersensitivity as a possible cause of rheumatoid arthritis, and this research effort is showing increasing promise. In gout treatment, new and more effective drugs are available and scientists have been able to further clarify the basic nature of the disorder. Diabetes research continues to feel the impact of the oral antidiabetic drugs and possible new uses for them have been investigated. Accomplishments in basic research were highlighted this year by the awarding of the Nobel Prize in medicine for work on the synthesis of nucleic acids. One of the recipients was a former chief of one of the Institute's laboratories, and the other was a grantee of the Institute.

In addition to its original three areas of special interest--rheumatic diseases, diabetes and basic metabolic research--the Institute has now expanded its scientific programs to include such new fields of interest as gastroenterology, cystic fibrosis and physical biology.

The advances to be described have resulted from work done both in the Institute's own laboratories and in the many non-government research centers throughout the country receiving support from the Institute in the form of research grants, training grants, traineeships and fellowships.

RHEUMATIC DISEASES

Only slightly more than ten years ago, just prior to the momentous discovery of cortisone's effects, the rheumatic disease field was one of the most neglected areas in medicine. For hundreds of years there had been little real progress in either understanding or treating rheumatoid arthritis, osteoarthritis, gout and the other rheumatic diseases that afflict more than 10 million people in this country. Most physicians shared the sentiment once expressed by the great Dr. William Osler--"When an arthritic comes in the front door, I want to go out the back door."

This discouraging situation can be contrasted with the very hopeful one that exists today. New and more effective anti-rheumatic drugs are now under constant development and have largely replaced cortisone in the treatment of rheumatoid arthritis. The Institute is continuing to place heavy emphasis on basic research, both intramurally and extramurally, and many important developments have been reported. Many of the biological details of these diseases have now been uncovered, explaining not only how the diseases affect the functioning of whole systems within the body but also how they affect individual cells, causing subtle but very important changes in metabolic processes. Some of the most intriguing work is being done on hypersensitivity as a possible cause of rheumatoid arthritis. Research in this area has begun to show increasing promise, although many of the findings are still inconclusive.

RHEUMATOID FACTOR FOUND IN HUMAN TISSUE FOR FIRST TIME

Rheumatoid factor, a substance known to occur in the blood of patients with rheumatoid arthritis, has now been discovered in two types of body cells: the plasma cells of joint membranes, lymph nodes and subcutaneous nodules and "large pale cells" in germinal centers of lymph nodes. The discovery of the factor in tissue cells as well as blood provides further clues about the nature of this puzzling substance and may lead to an improved diagnostic test.

Although considerable research has been done on the rheumatoid factor, and its presence in the blood is considered to be diagnostic of rheumatoid arthritis, the exact role it plays in the disease is still obscure. Studies have shown that the factor has many if not all the characteristics of an antibody, a protein substance which in some instances provides immunity to a particular disease, such as diphtheria, measles etc., and in other instances is the basis for a hypersensitive state such as hay fever, asthma etc. In rheumatoid arthritis this raises the

intriguing possibility that there is some antigen present, some deleterious substance to which the body is sensitive and against which it has produced the antibodies.

This theory has now been further substantiated by the discovery of rheumatoid factor in the plasma cells and germinal-center cells, since both of these cell types are usually engaged in antibody production. Studies indicated that the factor was not only present in these cells but also was actually produced in them. The factor was made brilliantly visible with the aid of fluorescein, a luminous yellow-green dye, and was detected only in cells taken from patients with rheumatoid arthritis. Further work with the dye technique may provide a more specific diagnostic test for the disease, and one which would be useful earlier in its course than are presently-used diagnostic blood tests.

(Dr. Robert C. Mellors and associates, The Hospital for Special Surgery, New York City)

In a disease closely related to rheumatoid arthritis namely Sjogren's syndrome, Institute scientists have recently discovered an unusually high incidence of diverse antibodies as well as abnormally elevated gamma globulins. This finding suggests that these rheumatic diseases may reflect a basic derangement in antibody production.

(Drs. Joseph J. Bunim and Kurt J. Block, Arthritis and Rheumatism Branch)

SENSITIVE NEW TEST MEASURES ARTHRITIS-PRODUCING ACID

Seeking clues to the mystery of arthritis, Institute scientists have developed a new

method for detecting and accurately measuring homogentisic acid, a substance which frequently causes a form of arthritis in persons with the rare metabolic disease alcaptonuria.

In 1957 scientists at the Institute first demonstrated that alcaptonuria is another disease that results from an inborn error of metabolism--the lack of a single enzyme, homogentisic acid oxidase. One of the characteristics of the disorder is the accumulation of homogentisic acid in the body. Deposits of this excess acid in cartilage cause a condition known as ochronosis. The disease is of particular interest to arthritis research because by the fourth decade of life most patients develop crippling ochronotic arthritis. This condition involves chiefly the intervertebral discs of the spine and the cartilages lining the knees, hips and shoulders.

The new laboratory technique for detecting and measuring homogentisic acid in blood and body tissues is more sensitive and more specific than older methods, and will be useful in detailed

studies of how the acid is related to the arthritic changes. Fundamental knowledge of how this one type of arthritis is produced may then help to explain the basic causes of other types of arthritis in which no specific causative agents have yet been found.

(Dr. Bert N. LaDu and associates, Arthritis and Rheumatism Branch)

EXPERIMENTAL GOUT DRUG SLOWS OVERPRODUCTION OF URIC ACID

Institute scientists have found an experimental drug, DON, which can slow down the

body's overproduction of uric acid, a common feature of gout. Although the drug produces undesirable side effects and therefore has little practical value, it points the way toward the development of similar drugs which will accomplish the same result and be non-toxic.

One of the characteristics of gout is a high level of uric acid in the blood. This is associated with recurrent attacks of arthritis and with deposits of uric acid crystals in the tissues and joints. These crystal deposits often destroy bone and damage tissue (such as kidney) as well as body surfaces by forming "gouty tophi" or nodules, particularly in joints, bone, kidneys, skin and ears. Treatment for the metabolic disease has included the administration of colchicine to overcome acute attacks, the reduced intake of certain foods, and the use of uricosuric (uric acid-removing) drugs to prevent deposit of the uric acid crystals which form the tophi. These drugs, however, do not correct the basic metabolic fault, which is the overproduction of uric acid.

The scientists found that DON, an experimental compound whose chemical name is 6-diazo-5-oxo-L-norleucine, caused a sharp fall in the blood level of uric acid and a drop in the production of uric acid in some gout patients. Isotope studies showed that the drug blocked an enzyme needed for the synthesis of new uric acid in the body. DON was originally found in a microorganism where it demonstrated antibiotic properties. It was subsequently synthesized by Parke, Davis and Company and is being used in cancer research as a tumor-inhibiting agent.

Because of the drug's ineffectiveness in some gout patients and its undesirable side effects, DON itself will not be practical in the treatment of gout. But it has demonstrated that uric acid production can be inhibited by drug therapy, and research is underway on similar drugs which can duplicate DON's enzyme-blocking action without causing any ill effects.

(Dr. J. E. Seegmiller, Arthritis and Rheumatism Branch)

SCIENTISTS FIND A CONNECTION BETWEEN GOUT AND GREATNESS

Evidence that gout, a metabolic disease associated with increased amounts of

uric acid in the blood, might be associated with greatness has been found by Institute scientists.

It has appeared to many students of the disease that the number of prominent scientists, statesmen, political and religious leaders, and writers who have suffered from gout is remarkably high considering the relative rarity of the disease. Among the great men of history who reputedly suffered from gout are Benjamin Franklin, William Harvey, Sir Isaac Newton, Charles Darwin, Martin Luther, John Calvin, Goethe, Alfred Lord Tennyson and Stendhal.

In an attempt to learn whether there actually is a connection, the investigators measured the levels of uric acid in blood taken from 817 successive inductees in the Armed Forces at Fort Dix, New Jersey and compared these with scores made by the same inductees on the Army Classification Battery, a group of tests designed to measure general intelligence and special aptitudes. The comparison of the scores on the test with the levels of uric acid showed that the higher the uric acid level, the more likely it was that the individual had a higher-than-normal score on the test.

Two interpretations can be given to the study, according to the researchers. One is that increased amounts of uric acid in the blood tend to promote intellectual achievement. The second is that higher level of uric acid in some individuals may reflect merely a diet richer in protein and purines, and bear no relation to intellectual functioning, although an attempt was made to eliminate this possibility by restricting the study to inductees who were on a uniform diet for 48 hours before the sampling. The study thus has afforded at least a partial scientific confirmation of the long-suspected association between gout and greatness.

(Drs. DeWitt Stetten, Jr., and John Hearon, Office of the Associate Director in Charge of Research)

HORMONE-CARRYING PROTEIN FOUND IN BLOOD PLASMA

A special protein in human blood plasma that is believed to play an important role in

transporting certain hormones throughout the body has been found by scientists at the Roswell Park Memorial Institute. The new protein has been named transcortin, since it binds hydrocortisone and corticosterone, two steroid hormones produced by the adrenal gland.

The binding of steroids by proteins in plasma has been known to exist for some time and is considered to be part of the mechanism for transporting hormones in the body. In recent years considerable research has been done to determine how specific this binding is, how much of it takes place, and how tightly bound the steroids and the proteins become. Although a great deal is known about many of the steroid hormones, relatively little has been learned about the plasma proteins; most of them have never been isolated.

The Roswell Park scientists, extending earlier studies by another research group that suggested the existence of transcortin, were able to separate it from the bulk of the plasma proteins and to purify it 150 times. They believe that the hormones are biologically inactive when bound by transcortin, hormonal activity being possible only when there are more hormones present in the blood than can be bound by the available protein.

(Drs. W. Roy Slaunwhite, Jr., and Avery A. Sandberg, Roswell Park Memorial Institute, Buffalo, New York)

DROOPING RABBIT EARS DEMONSTRATE EFFECTS OF STEROIDS ON CARTILAGE Sad-looking rabbits with a hound-dog aspect, their ears drooping much as do those of beagles and bloodhounds, played an important role in a research project conducted by Institute grantees at New York University-Bellevue Medical Center. Studies of the rabbits, whose ears had been temporarily "collapsed" by injections of papain, have produced new knowledge concerning the action and effects of steroid such as cortisone, hydrocortisone and prednisolone, hormones widely used in the treatment of rheumatic diseases.

Young rabbits used in the study were given injections of papain, an enzyme extracted from the fruit of the tropical papaya plant. Papain, more commonly used in homes and restaurants as a meat tenderizer, causes basic changes in the rabbits' body cartilage and results in progressive collapse of the rabbits' normally erect ears. Ordinarily, in living rabbits, cartilage restoration takes place quickly after injections of the enzyme, the ears recovering their usual tone and erect state within three to five days. It was found, however, that the ears would remain collapsed--indicating interference with cartilage recovery--if daily injections of cortisone, hydrocortisone or prednisolone were given. Once the steroid injections were discontinued the ears became erect again.

Other work with these experimental animals indicated that the action of the steroids in preventing normal cartilage

recovery was due to direct effect on cartilage rather than the result of a general metabolic or systemic effect.
(Drs. R. T. McCluskey and Lewis Thomas, New York University-Bellevue Medical Center, New York, N. Y.)

RESEARCHERS FIND THAT HORMONES INHIBIT ACTION IN BODY CELLS

Institute scientists, exploring the basic nature and varied activities of

steroid hormones in the body, have found that a number of them are potent inhibitors of one of the key metabolic reactions taking place within the living cell--the oxidation of the substance known as DPNH, or diphosphopyridine nucleotide.

DPNH, in its oxidized form, DPN, makes possible a vital step in the complicated metabolic process by which food substances are converted into energy in the body. DPN is a co-factor required by enzymes for the oxidation or "burning" of food within the cell. During this process DPN itself is changed into DPNH and is then, in the normal course of things, changed back into DPNH so that it may again play its part in the process. It is at this point, the NIAMD scientists found, that the steroid hormones inhibit the action, interfering with the process by which DPN is reconverted to DPNH, thus breaking the chain of reactions.

Among the steroids found to inhibit this important step in the metabolic processes were cortisone, dihydrocortisone, corticosterone, testosterone, progesterone, estradiol and diethylstilbesterol. The biological significance of the finding has yet to be fully evaluated, for the role of the steroid hormones in the regulation of cell metabolism is not yet well understood. This newly found specific role of certain steroid hormones in relation to the oxidation process within the cell will be the subject of further effects and another possible step toward the better understanding of basic life processes.

(Drs. K. L. Yielding, Arthritis and Rheumatism Branch, and G. M. Tomkins, Laboratory of Biochemistry and Metabolism.)

DIABETES

Diabetes is probably the best known and most important of the metabolic diseases. It results from either an insufficient production of insulin by the pancreas, or from interference with insulin's action after it has been produced. Because of this abnormality, the diabetic patient is unable to properly utilize sugar (glucose) and excess amounts of it build up in the blood and spill over into the urine. It is an extremely complex disorder which is now known to encompass alterations in fat and protein metabolism as well as sugar metabolism.

There is still much to be learned about basic mechanisms that are operating in this common metabolic disease. More research on the spatial configuration and function of insulin is needed, and because of many interrelationships, the biochemistry and metabolism of other regulators--especially the hormones of the pituitary and adrenal glands--must be further investigated.

The recent widespread use of the new oral antidiabetic drugs has brought about major changes in the treatment of thousands of diabetics who have, under medical supervision, exchanged their regular insulin injections for one of the new tablets. Their ultimate value in the treatment of diabetes remains to be seen but at the very least they have played a valuable part in fostering a dramatic new surge of scientific interest in an age old disease.

CONTINUING USE OF ORAL ANTIDIABETIC DRUGS HELPS TO CLARIFY THEIR VALUE Three years of experience with the new oral antidiabetic drugs have helped to clarify their value in the treatment of diabetes. The first of these blood sugar-lowering compounds became available in June, 1957. This was tolbutamide (Orinase) and it was soon followed by two others, chlorpropamide (Diabinese) and phenformin (DBI). Although these new drugs are useful in a great many diabetics, it is now apparent that they have definite limitations and must be used only under careful medical supervision.

The oral drugs have freed thousands of diabetics from their dependence on regular injections of insulin, but it must be emphasized that they are not "miracle drugs." They do not "cure" diabetes nor do they replace insulin in the body. As

far as is known at the present time their major action is to encourage the still-active insulin-producing parts of the pancreas to secrete larger quantities of this essential hormone.

Tolbutamide and chlorpropamide are useful, in the main, only to a selected group of middle aged diabetics and even in these cases they do not eliminate the need for carefully regulating the diet. These two drugs are not likely to be effective in diabetics who can produce little or no insulin; few young diabetics can use them and almost no adult "brittle" or "juvenile" diabetics can use them.

The newest of the oral drugs now available, phenformin, does act in some "juvenile" diabetics but experience with it is limited and much longer-term trial is necessary to determine how effective it is in these patients. Longer-term trials are needed with the other drugs, also, since their full value will not have been conclusively proved until the lifetime experiences of hundreds of diabetics can be studied. Of considerable interest is whether or not some of the complications that often accompany diabetes, such as retinopathy, will be any more or less frequent in diabetic patients receiving the oral drugs instead of insulin.

One promising application of the oral drugs is in diabetes prevention. Using a small group of individuals who were not frankly diabetic but who were considered "diabetes susceptible" Institute grantees at the University of Michigan have tested the glucose tolerance curves (an indication of sugar utilization in the body) before and after long-sustained treatment with an oral drug and have found some improvement in these curves. It is much too early to determine the true significance of this finding but it suggests that the oral drugs may have a part to play in the prevention of diabetes in susceptible individuals.

Unquestionably, the most important result of the discovery of the oral antidiabetic drugs--limited though they are--has been their encouragement of new research on diabetes. Studies by scientists at the Institute and grantees at non-federal research centers are providing valuable information about carbohydrate metabolism and are re-kindling the scientific interest that may usher in a new era in the prevention, control and cure of diabetes.

GENETICS UNABLE TO PREDICT AGE OF ONSET IN DIABETES

one member of the same family, but it was not until 25 years ago that the genetic component of this disease was firmly established. Since then, researchers studying family histories

It has been known for hundreds of years that diabetes frequently occurs in more than

have attempted to predict the age at onset of diabetes in children of diabetic parents, hoping to be able to say that after a given age the risk for any particular person would be essentially nil. An early study suggested that the average age at onset decreases 20 years per generation, and that a child rarely if ever becomes diabetic at a greater age than did his parent.

More recently, however, researchers at Western Reserve University have shown that the parent's age at onset cannot be used to predict either the child's age at onset, or the end of the period of risk for the child. The scientists studied 301 pairs of diabetic parents and children and found that although the majority of children had become diabetic at an earlier age than had their parents, the number was no greater than that expected to occur by chance alone, when no biological relationship is assumed to exist between the age of onset in the parent and in the child.

(Dr. Arthur G. Steinberg, Western Reserve University, Cleveland, Ohio)

STUDY SHOWS LOW FAT DIET MAY BENEFIT DIABETICS

A study of eleven diabetic patients kept on a strict low fat diet for one to two

years has shown that this type of diet can lower the amount of lipids (fatty substances) in the blood, increase the body's ability to handle excess sugar, and benefit the retinopathy (eye disease) that often accompanies diabetes and leads to blindness.

During the study, diabetic patients were kept on a diet containing only 20 grams of fat daily in contrast to the average diabetic diet of 80 to 110 and the average normal intake of 135-175 grams of fat per day. At the beginning of the study all the subjects had elevated amounts of lipids in their blood as well as symptoms of retinopathy. Some were taking insulin to control the diabetes while others could control the disease by diet alone; most had been diabetic for more than ten years.

The low fat diet produced a striking increase in the patients' ability to tolerate blood sugar, making it possible to increase dietary carbohydrates to at least 135 grams without increasing the amount of insulin needed. The same dietary change could be made successfully in patients who did not require insulin. Studies of the blood lipids, done three, six and twelve months after the diet was started, showed that lipids decreased in 10 of the 11 patients who followed the diet. Blood cholesterol levels dropped 20 percent.

Most of the patients also showed beneficial changes in their retinopathy, a disease in which the retina of the eye becomes damaged and vision is impaired. One of the characteristics of this disease is the leaking of material--exudates--from the small blood vessels in the retina. The chemical composition of these exudates is unknown, but it seems possible that they contain lipids. In 10 of the patients with exudates, a marked decrease or disappearance occurred in five and a small but definite reduction occurred in three others. Although the study suggests a relationship between the lowering of the blood lipids and the disappearance of exudates, further research will be necessary to confirm this finding.

(Dr. W. F. Van Eck, Yale University School of Medicine, New Haven, Conn.)

FAT TISSUE MAY BE MAJOR SITE OF INSULIN ACTION

Scientists have found that fat tissue, far from being merely a storage place for

body fat, may well be a major site of insulin action in the body. This and other recent findings have led to a reappraisal of the metabolic importance of adipose (fat) tissue and have indicated that it is an extremely active system which is intimately concerned with the synthesis, oxidation and release of body fats.

Studies were done with radioactive compounds to measure insulin's effects on adipose tissue taken from rats. They showed that the addition of insulin to this tissue increased the rate of sugar (glucose) metabolism six times, and caused a ten-fold increase in the rate of fatty acid synthesis. These stimulatory effects were produced with extremely small amounts of insulin, amounts that might normally be present in the tissue.

The findings support the current concept that one of insulin's primary functions is to make glucose from outside the cell available for intracellular metabolism. Adipose tissue's marked sensitivity to insulin, in addition to its rich blood supply and relatively great bulk, suggest that it is a major, if not the major, site of the hormone's action.

(Dr. G. F. Cahill, Jr., and associates, Harvard Medical School and Peter Bent Brigham Hospital)

CONDITIONING AFFECTS BODY'S ABILITY TO UTILIZE SUGAR

The surprising response of a professional roller skater to a glucose tolerance test

started Institute scientists on a study which showed that both physical conditioning and prolonged bed rest have profound effects on how well the body utilizes this sugar, a primary source of energy.

The skater, a 20-year-old normal "control" patient, was given a standard glucose tolerance test during the course of other studies at the Institute. This test, which determines how well the body handles sugar, consists of administering a known amount of glucose while the subject is at rest, and then measuring the change in the blood sugar level. Normally, the level increases markedly at first and then gradually returns to its original value, but in the case of the skater there was no detectable change in blood sugar level. This indicated an amazingly fast turnover of the excess sugar, which was either being consumed immediately, or stored by the body in the form of glycogen.

The skater reported that while skating professionally she often practiced all day without food and then continued to skate at night in roller derbies without eating. This led the investigators to speculate that the skater's physical conditioning had resulted in the excellent utilization of food energy. They studied the phenomenon by putting eight other normal control patients on a two-week regimen of enforced bed rest followed by a two-week program of exercise. Glucose tolerance tests were given during each period and showed that the prolonged bed rest caused a marked decrease in blood sugar utilization, resulting in blood sugar levels in the diabetic range. A week after the activity program began, however, utilization returned to its original value in all the subjects and even higher in some.

These different effects of bed rest and physical activity on glucose tolerance may explain why chronically ill persons often give a diabetic response to this test and why diabetics sometimes improve on a regimen of exercise. Further studies in the Institute's specially-constructed metabolic chamber, planned to uncover the underlying mechanisms, are under way. (Drs. Leo Lutwak and G. Donald Whedon, Metabolic Diseases Branch)

METABOLIC DISEASES

The metabolic diseases are caused by errors or defects in metabolism, the basic life process by which the body converts air, food and water into energy, and by which growth and replacement of tissue constituents are made possible. They are diseases in which something has happened to the normal relationship that exists between hormones, vitamins and the hundreds of enzymes in the human body.

Among the metabolic diseases, the "molecular" diseases are of particular interest. These so-called inborn errors of metabolism are the result of a partial or total lack of a specific enzyme necessary to catalyze a vital metabolic process. The disorders are hereditary in nature because the biosynthesis of the missing molecules (enzymes, etc.) is under genetic control. The study of these diseases presents many problems: the deficient molecule must be identified, the biochemical consequences of its deficiency determined, the hereditary transmission of the defect clarified and possible forms of treatment explored.

A striking example of a molecular disease that has been of interest to scientists at the Institute is galactosemia, and research during the past year has raised the possibility that such an inherited defect can be overcome.

RAPID TEST DEVELOPED FOR DIAGNOSING PKU AT BIRTH

A simple and rapid blood test
for diagnosing the metabolic
disease known as phenylketonuria

(PKU) has been developed at the Institute. The test now makes practical the diagnosis of the disease within a day after birth, and will aid physicians in controlling the special diet vitally needed by PKU patients to avoid mental retardation.

Phenylketonuria is an "inborn error of metabolism" whose most disastrous consequence is severe mental retardation. The disorder is caused by the lack of a single enzyme in the body, an enzyme needed to convert (metabolize) phenylalanine, one of the amino acids, into tyrosine, another amino acid. Because the enzyme is lacking, this metabolic "step" is blocked and abnormal amounts of phenylalanine accumulate in the blood. Although there

is no cure for the basic defect, it has been found that a low-phenylalanine diet may prevent some if not all of the mental retardation. Rapid diagnosis is crucial, for the earlier the diet is started--within days or weeks after birth--the better the chances are for normal mental development.

The new diagnostic test is relatively simple and can be performed within ten minutes, whereas the older methods were complex and time consuming. A great advantage of the test is that it can be performed on finger-prick samples of blood and thus is practical to use in infants. Another advantage is that the new test not only measures phenylalanine, but also measures the level of the amino acids tyrosine and tryptophan in the blood. This is important during treatment with the PKU diet, for tyrosine must be added in the correct amounts to supply what normally would be produced by phenylalanine metabolism.

(Dr. Bert LaDu, Arthritis and Rheumatism Branch.)

HORMONE INJECTIONS HELP OVERCOME METABOLIC BLOCK

Injectations of progesterone, the female sex hormone, in patients with the metabolic

disease galactosemia have shown that it may be possible to circumvent the dangerous enzymatic "block" that causes the disorder. This finding not only holds the promise of improved treatment but may also set an important precedent for research in other disorders caused by enzyme deficiencies.

Galactosemia is a relatively rare hereditary disorder of children. Afflicted infants are unable to tolerate galactose, a sugar present in milk, because they lack one of the enzymes necessary to metabolize the sugar. If milk is allowed to remain in the diet of a galactosemic patient it quickly creates a toxic condition leading to mental retardation, cataract formation, liver damage and early death. The biochemical cause of the disease, unknown prior to 1955, was discovered in that year by Institute scientists who isolated and identified the missing enzyme.

In the recent studies, scientists found that injections of progesterone over a six-day period enabled three young galactosemic patients to metabolize at least part of an intravenous dose of galactose. Before receiving the hormone injections, they could metabolize none of the sugar. The hormone evidently enabled the body to circumvent the block caused by the missing enzyme, a block which had previously been completely effective. Although the exact mechanisms responsible for this hormone effect are not yet understood, the important aspect of the study is that

it raises the hope that by one means or another, enzymatic blocks causing other metabolic diseases can be overcome. (Drs. Y. J. Topper, L. A. Pesch and E. R. Simon, Laboratory of Biochemistry and Metabolism, and Dr. S. Segal, Clinical Endocrinology Branch)

RESEARCH REVEALS IDENTITY OF IRON-RELEASING ENZYME

Scientists have now identified, for the first time, a specific biochemical mechanism for the movement of iron in the body. They have demonstrated in animals that iron, needed for the hemoglobin in red blood cells, is released from the liver by the action of an enzyme (xanthine oxidase).

Iron occurs in the liver in the form of ferritin iron, a combination of iron and protein which is believed to be the storage form of the element in the body. Earlier test tube studies had shown that the enzyme acts on this combination to change the iron into a form which makes it available for removal into the circulating blood. The more recent study confirms that this reaction takes place in the same manner in the living guinea pig, rabbit and dog.

This enzymatic reaction appears to be part of a body mechanism for regulating the level of iron in the circulation. When bleeding occurs, the blood supply to the tissues is disrupted and produces a condition known as hypoxia, or oxygen-lack, in the tissues. The investigators found that when hypoxia occurs, the enzymatic reaction in the liver speeds up, releasing more iron into the blood. This increased supply of iron is then used by the bone marrow to produce more oxygen-carrying red blood cells. As the new supply of red cells reaches the tissues, the hypoxia is corrected and the enzymatic reaction slows down again to its normal rate.

(Drs. A. Mazur and associates, Cornell Medical College and New York Hospital, New York, N. Y.)

RESEARCH WORKERS GAIN NEW KNOWLEDGE OF VITAMIN D ACTION

The use of vitamin D for the treatment of rickets, a deficiency disease of bone metabolism, is well established, but there is still little fundamental knowledge of the biochemical or enzymatic ways in which the vitamin operates. Recent studies by scientists at Johns Hopkins University now shed new light on the vitamin's activity and the manner in which it is transported by the blood.

The investigators were able to measure experimentally the anti-ricketic (vitamin D) activity in blood serum, finding that in normal individuals this activity was equal to two International

Units of vitamin D per milliliter of serum. This amount did not increase unless large doses of the vitamin were ingested. Studies of the physical properties of the antiricketic substance indicated that it was evidently bound to large molecules in the serum, since all vitamin D activity was lost if the samples were subjected to exceedingly fine filtration.

These observations have been clinically helpful in the investigation of a number of disorders of calcium metabolism and provide some insight into the variable responses encountered in patients treated with vitamin D.

(Dr. William C. Thomas Jr., and associates, Johns Hopkins University and Hospital, Baltimore, Maryland)

NO HARM FOUND IN DIETS CONTAINING EXCESS MILK

Recently expressed fears that diets containing too much milk may be harmful have not

been supported by scientific evidence. On the contrary, it now appears that high calcium diets may even be necessary in some elderly patients to maintain normal bone structure.

The adult daily requirement of calcium, based on studies in healthy, relatively young adults, is between 450 and 600 milligrams, an amount supplied by one pint of milk. Intake of greater amounts is considered necessary during growth, pregnancy and lactation, but it has been feared that during other periods of life larger amounts of milk might be damaging. Some researchers have suspected that high calcium intakes might cause calcium to be deposited in soft tissue, or calcium stones to form in the urinary tract.

A review of the many studies done to date reveals that there is no evidence available to support the belief that high calcium intakes alone can affect the calcium content of either blood or soft tissues. Those diseases in which calcium is deposited in muscle or under the skin apparently involve abnormal calcium metabolism in the body and are not caused by the excess calcium. Urological studies have shown that high calcium intakes do not play a significant role in the formation of urinary tract stones; in fact, some experimental stone studies in animals indicate that high dietary calcium may actually decrease stone development. A high level of calcium in the diet appears to be beneficial in at least one disease: osteoporosis, a disorder of aging in which the bones become thin and fragile. Recent metabolic balance studies of five such patients at the Institute showed that they required an average of 1100 milligrams of calcium per day just to supply body needs.

(Dr. G. Donald Whedon, Metabolic Diseases Branch)

BODY'S SALT AND WATER BALANCE CONTROLLED BY BRAIN SUBSTANCE

New knowledge of how the body regulates the important balance between salt and

water has been provided by studies of aldosterone, the powerful sodium-regulating hormone produced by the adrenal gland. Researchers at Western Reserve University have recently found evidence that the adrenal's secretion of aldosterone is stimulated by a specific substance which is produced in the brain and reaches the adrenal via the bloodstream.

Although isolated less than ten years ago, aldosterone is now known to be the most important salt and potassium regulating hormone in the body, bringing about the retention of sodium and the excretion of potassium and water by its action on the kidneys. It is secreted by the part of the adrenal gland known as the zona glomerulosa, but the factors which control the exact amount of hormone secreted have been unknown. Earlier studies by other investigators had shown that the glands are not dependent upon nerve supply for aldosterone output, nor is the zona glomerulosa controlled by the body's master gland, the pituitary.

In attempts to isolate a stimulating factor, the researchers made extracts of various brain tissues and tested their effects on the adrenal gland. They found that extracts from the posterior diencephalon or "interbrain" were most effective in stimulating aldosterone secretion, and tentatively named the new factor glomerulotropin, since its target organ is presumably the zona glomerulosa. Further studies are being done to actually isolate the substance and determine the exact area in the brain where it originates.

(Dr. Gordon Farrell, Western Reserve University, Cleveland, Ohio)

GASTROENTEROLOGY

Research in gastroenterology is just beginning to feel the impact of the Institute's expanded program of support, but substantial contributions have already been made in this long-neglected field. More and more investigators are becoming interested in the diseases of the digestive system (peptic ulcer, ulcerative colitis, ileitis and others, and new research techniques have been developed.)

During the past year Institute-supported research in non-federal research centers has provided many new findings, some of which are readily applicable to the treatment of the diseases themselves and others which provide more fundamental knowledge about organ function and biochemistry.

At the Institute's clinical facilities in Bethesda a new gastroenterological unit has been established to carry out intensive studies of the diseases which are grouped under the term "mal-absorption syndrome." These diseases are ones in which the absorptive capacity of the small intestine has been affected. One of the primary aims of the new unit is to apply the new knowledge of biochemistry to the study of the digestive organs, investigating the metabolic processes that transpire in the cells lining the stomach, intestines and gall bladder so that these processes can be related to physiological activity.

ULCERATIVE "COLITIS" INDUCED IN RABBITS TO AID RESEARCH WORK A condition that resembles ulcerative colitis in humans has been produced in rabbits by using immunologic techniques. This research accomplishment lends further support to the belief that ulcerative colitis involves some type of antigen-antibody reaction, although the evidence is still not conclusive.

Ulcerative colitis is a disease primarily affecting the large intestine, or colon, and is characterized by bleeding, inflammation and ulceration of the colon's lining. Since the disease does not occur naturally in animals, numerous attempts, only partially successful, have been made in the past to induce it experimentally. The present work was based on the theory that

inflamed tissue in the body has an attraction for circulating antigen and antibody. (Antigens are foreign substances to which the body becomes sensitized, and against which it produces antibodies.) The researchers reasoned that by causing a mild inflammation of the colonic tissue in sensitized rabbits they might be able to produce a state of local hypersensitivity in the colon, a "colitis."

The rabbits were first sensitized to a specific antigen, egg albumin, to produce a generalized antibody response, and their colonic tissue was mildly irritated with a weak solution of formalin. Then a second or "challenging" dose of egg albumin was injected in the hope that the resulting antigen-antibody reaction would be localized in the inflamed colon and produce "colitis." The experimental technique, known as the Auer procedure, did cause inflammation of the colon, ulceration and other characteristic signs of ulcerative colitis, but whether the antigen-antibody reaction was the actual basis of the "colitis" has yet to be conclusively determined. Further studies are being done with the technique and will contribute to a better understanding of the reaction of the colon to injury in general, and perhaps to the cause of ulcerative colitis. (Dr. J. B. Kirsner and associates, University of Chicago.)

NEW RADIOACTIVE TECHNIQUE TESTED IN ULCERATIVE COLITIS STUDIES A new method for measuring absorption in the colon which may prove of great value in the study of ulcerative colitis and other gastrointestinal diseases, has been developed by scientists at the Duke University Medical Center. Small amounts of radioactively-labeled substances are added to the barium enema routinely used in X-ray examination of the colon. The amount of absorption is determined by measuring the radioactivity of blood samples taken at intervals after the procedure, since the absorbed material passes through the colon and into the bloodstream.

Although the large bowel or colon has long been considered principally a collecting and dehydrating segment of the intestine, in recent years it has been shown that a certain amount of absorption does take place there. The investigators reasoned that if this absorption could be measured, it might provide a useful index of the condition of the colon's lining. This would be of great value in the management of patients with gastrointestinal diseases such as ulcerative colitis, where large areas of the colon may become eroded.

Various radioactively-labeled substances were tested, including fats and proteins, but the only one that was absorbed to any measurable extent was sodium iodide. Studies done in both

normal individuals and in patients with ulcerative colitis showed that considerably more of the radioactive sodium iodide was absorbed by the normal individuals than by the ulcerative colitis patients. Follow-up studies are being done to determine more fully the value of the technique.

(Dr. J. K. Isley and associates, Duke University Medical Center, Durham, N. C.)

SURGEONS FIND BILE "BACKFLOW" MAY BE CAUSE OF PANCREATITIS

Investigators studying the severely painful and sometimes fatal disease, pancreatitis,

have shown that it can occur when a combination of pancreatic secretions and bile "back up" into the pancreas. The presence of this mixture in the pancreas then causes the destruction of parts of the organ through enzymatic digestion. The careful study, done in animals, has now provided an answer to a problem which has baffled pathologists for years.

The protein-digesting enzymes present in bile have long been suspected as the cause of pancreatitis. This is because the main bile duct, from the liver, usually joins the principal duct from the pancreas and forms a common duct into the intestine. It has been suspected that under high enough pressure, the bile might be forced back up the common duct, through the pancreatic duct and into the pancreas. Previous investigators have found, however, that this sequence of events happens only under considerable pressure, much higher than would normally occur.

Surgical researchers at Ohio State University have now been able to show that backflow into the pancreas is possible under normal pressure. The gall bladder stores bile from the liver, and might become filled with the mixture if a gallstone happened to block the common duct, creating a backflow. Inside the gall bladder, a new and destructive mixture is formed which then flows into the common duct and is forced into the pancreas. Although the pancreas would reject the entrance of bile alone, it readily accepts this mixture of bile and its own secretions, and thus permits the onset of its own destruction. Patients with this disease are aided by surgical opening of the bile ducts, but further surgical research will be needed to determine the best form of treatment.

(Drs. D. W. Elliott, R. C. Williams and R. M. Zollinger, Ohio State University.)

ALCOHOLISM NOT SOLE CAUSE OF CIRRHOSIS OF THE LIVER

A comprehensive review of the scientific evidence that links alcohol to liver injury indicates

that neither chronic alcoholism nor the malnutrition associated with heavy drinking can be considered the sole cause of cirrhosis of the liver.

Alcoholic cirrhosis, or Laennec's cirrhosis, is a chronic disease in which there is an infiltration of fatty tissue in the liver, followed by an increase in connective tissue. These changes distort the normal architecture of the liver and impair its function, ultimately causing jaundice, swelling and circulatory disturbances.

The relationship between chronic alcoholism and cirrhosis has been recognized for over a century and is supported by autopsy evidence indicating that the incidence of cirrhosis in alcoholics is at least six times as high as that in non-alcoholics. Although it is generally believed that cirrhosis is associated with the faulty dietary habits that often accompany habitual drinking (in particular the dietary deficiency of choline, a member of the vitamin B complex) it is becoming increasingly evident that other deficiencies may be involved.

Recent evidence indicates that it is possible that alcohol affects the liver in subtle ways, such as by interfering with some of the organ's enzymatic activities. Also, certain features of cirrhosis in alcoholics suggest that the liver may be the site of a bacterial infection, although bacterial cultures are usually inconclusive or negative. Another more interesting possibility is that the hepatitis virus is implicated and that chronic alcoholism either increases the susceptibility to infections or activates latent infections in carriers.

AMMONIA INTOXICATION CLEARED BY AMINO ACID INJECTION

Ammonia intoxication, a critical condition caused by high levels of ammonia in the blood, has been successfully treated with arginine, an amino acid. Physicians at the University of California School of Medicine have demonstrated that arginine, when administered intravenously, can bring about the rapid lowering of the blood ammonia level by increasing the body's production of urea. This ammonia-containing compound is then eliminated in the urine.

Normally, ammonia is produced in the gastrointestinal tract by the action of bacteria on ingested protein substances and is made nontoxic by biochemical reactions in the liver. The action of the normal liver is so efficient that usually little or no ammonia escapes into the blood. However, ammonia accumulates in the blood when the liver becomes damaged from infection or such diseases as cirrhosis, or when there has been extensive gastrointestinal hemorrhage. When too much ammonia is circulating, some of it crosses the blood-brain barrier and causes mental confusion and other neurological signs which will progress to complete unconsciousness and death if not immediately removed.

The arginine therapy was used in 90 patients suffering from ammonia intoxication from various causes and brought about a prompt reduction in blood ammonia levels. This was usually followed by improvement in the patients' clinical condition. (Dr. J. S. Najarian and associates, University of California School of Medicine, San Francisco.)

NEW TECHNIQUE FINDS CAUSE OF SERIOUS INTERNAL BLEEDING

Surgical researchers have developed a new diagnostic method for quickly deter-

mining the cause of serious bleeding in the gastrointestinal tract. The technique is called splenic pulp manometry and consists of inserting a needle into the spleen to measure splenic blood pressure. If the pressure is abnormally high--over 290 millimeters of water--it indicates the presence of varices, distended veins in the area of the stomach and esophagus, that often rupture from high internal pressure and cause extensive hemorrhage.

Such massive hemorrhaging in this area is an emergency that often confronts the physician and demands immediate localization of the site of the bleeding. This is often an extremely difficult task since there are a number of possible causes of such bleeding. Most commonly the cause is a perforated ulcer, requiring emergency surgery. But, in about 25 percent of cases, ruptured varices are causing the bleeding and since these are treated non-surgically it is vitally important to quickly distinguish between the two causes. The new technique determines the presence or absence of varices with 90 percent accuracy.

In a study of 113 patients, the researchers found that all patients who either were bleeding or had bled from varices had splenic pressure readings of over 290 millimeters (mm) and if the pressure was below 250mm the bleeding in all instances had resulted from another cause. In the 11 patients who had pressure readings between 250 and 290mm, more intensive studies were necessary to determine the presence or absence of varices. However, in all the patients in this "overlap" group, the bleeding either stopped spontaneously or after non-surgical treatment. The safety of the new technique is indicated by the fact that there were no complications in this large series of cases. The immediate knowledge it provides results in more effective management of this type of medical emergency. (Dr. William Panke and associates, New York City)

DIET THERAPY SUCCESSFUL IN NON-TROPICAL SPRUE

It has now been demonstrated that consistent adherence to a diet completely free of

gluten, a cereal grain fraction, will aid in controlling the disease known as non-tropical sprue.

This disease is probably the largest entity in the group of disorders known as the malabsorption syndrome and is associated with a long-lasting debilitating diarrhea, weakness, weight loss and many other signs resulting from failure to absorb certain proteins. Sprue was first thought to be limited to tropical and subtropical latitudes, but is now known to exist in northern regions as well.

Working from earlier reports that gluten, one of the major fractions of cereal grains, might be the offending agent, a team of Institute grantees performed metabolic balance studies on sprue patients and found that eliminating gluten from their diet resulted in increased absorption of fat and important minerals, permitting replenishment of body protein stores. They then developed a special diet which contained unlimited fat, meat protein and vegetable fiber, but was free of all wheat, rye, oats and barley. When the gluten-free diet was used as the sole means of therapy in 20 patients with non-tropical sprue it brought about a sustained improvement in 17 of them. The lack of improvement in two of the remaining three patients was associated with failure to follow the diet. The gluten-free diet thus provides a practical method of treatment which can now be confidently recommended for patients with non-tropical sprue.

(Drs. M. H. Sleisenger and associates, New York Hospital-Cornell Medical Center, New York City)

INCREASED ENZYME ACTIVITY MAY SIGNAL GASTRIC CANCER

Studies of an enzyme called
lactic dehydrogenase (LD)
have shown that changes in

its level of activity in gastric juice may furnish a useful diagnostic aid in suspected cases of gastric cancer. The enzyme is essential to normal carbohydrate metabolism and is widely distributed in body tissues and fluids. But through some as yet unknown mechanism its level of activity in blood serum and body fluids varies in response to disease processes in the tissues.

When analyses were made of gastric juice samples taken from patients with known gastric cancer they were found to contain abnormally high levels of LD activity, averaging 910 units per cubic centimeter of gastric juice. This is in marked contrast to the LD activity of normal gastric juice which was found to be less than 200 units per cubic centimeter. It was also found that in the gastric cancer patients the LD activity was higher in the gastric juice than it was in the blood. This is the reverse of the situation found in normal persons where the LD activity is highest in the blood. The findings indicate that the test, when giving positive results, may be helpful in the diagnosis of gastric cancer, and further work is being done to increase its diagnostic usefulness.

(Dr. Steven Schenker, University of Cincinnati College of Medicine, Cincinnati, Ohio)

BASIC RESEARCH

Basic research continues to receive major emphasis in the studies conducted and supported by the Institute. The nature of the rheumatic diseases, diabetes and the other metabolic diseases makes this vitally necessary, for despite the real and gratifying progress made in the control of these diseases, we still know far too little about their fundamental cause and nature.

Accomplishments in basic research were highlighted this year by the awarding of the Nobel Prize in Medicine for work done on the synthesis of the nucleic acids. These acids are chemical substances inside the cell that are believed to control hereditary characteristics and because of their great fundamental importance the Institute has placed heavy emphasis on their study for the past several years. This year's recipients of the Nobel Prize were Dr. Arthur Kornberg, a former chief of one of the Institute's laboratories, now at Stanford University, and Dr. Severo Ochoa, an Institute grantee at New York University.

Like the nucleic acid research, many of the studies now being pursued have no immediate application to the treatment of any particular disease. Their implications, however, encompass all the medical sciences.

RARE EIGHT-CARBON SUGAR FOUND
IN NATURE FOR THE FIRST TIME

Institute scientists have discovered and isolated the first eight-carbon sugar to be found

in nature. The rare sugar, known as an octulose, has been found in both the California avocado and the sedum plant, and is now the highest carbon natural sugar known to exist.

Two of the most common sugars occurring in nature are glucose and fructose, which are six-carbon sugars. That is, they contain six carbon atoms in every molecule. Only two seven-carbon keto sugars, or heptuloses, have ever been found, and until the discovery of the octulose they were thought to be the highest carbon sugars in nature.

The isolation of the eight-carbon sugar, or octulose, resulted from a study of the California avocado, which was being analyzed for its sugar content. An unusual cherry red spot which appeared on the paper chromatograms being used in the analysis was the first clue to the presence of the rare sugar. The pulp from one hundred avocados was then thoroughly fractionated, yielding approximately one gram of the octulose. The scientists were also able to isolate an octulose from the sedum plant. This plant was chosen because, like the avocado, it was known to contain a seven-carbon sugar and thus might also contain sugars of even higher carbon content. Almost 400 pounds of the plant were needed to produce sixteen hundredths of a gram of the eight-carbon sugar. Analysis showed that it was identical to the octulose isolated from the avocado.

The biological importance of the rare sugar has yet to be determined. After the seven-carbon sugars were discovered in 1917 they remained laboratory curiosities until a few years ago. Then they suddenly came into prominence when an enzymologist working at the Institute discovered an enzyme in rat liver that could synthesize one of the heptuloses. Since then, heptulose has been found in beef liver and in almost all plants, where it participates in the early stages of photosynthesis. The new-found octulose may have an equally important role in plant and animal metabolism.

(Drs. A. J. Charlson and Nelson K. Richtmyer, Laboratory of Chemistry)

CHROMIUM MAY BE AN ESSENTIAL TRACE ELEMENT IN THE BODY

Chromium, not previously considered important in nutrition, has been found necessary in

rats to maintain a normal tolerance for blood sugar. The study raises the possibility that a similar requirement may exist in other mammals, including man, and that chromium may somehow be related to the impaired glucose tolerance that is found in diabetes and other diseases.

Discovery of the importance of chromium to the rat climaxes four years of study since 1955 when it was found that rats fed special diets were not able to rid themselves quickly of excess glucose (blood sugar) which had been injected into their blood streams. A dietary agent called the glucose tolerance factor was able to correct the condition and the element chromium has been found to be the active ingredient in this factor.

The importance of the factor in the rats' diet was demonstrated by the finding that glucose disappeared from the blood stream at the rate of 2.8 percent or less per minute in deficient-diet

experimental animals as compared with a rate of 4.0 percent per minute in animals fed a diet containing the necessary factor. Only one millionth of a gram of chromium daily is required to maintain normal glucose tolerance, but 20 times that amount is needed to correct an established deficiency. The scientists found that only the trivalent form of chromium--chromium (III)--is biologically effective and they believe that special mechanisms may be provided for within mammalian organisms to absorb and handle it.

(Drs. Klaus Schwarz and Walter Mertz, Laboratory of Nutrition and Endocrinology)

BACTERIA CAUSING RAT LEPROSY NOW GROWN IN TISSUE CULTURE

The bacteria which cause leprosy in rats have now been successfully grown in a special tissue culture developed at the Institute. The culture is able to maintain the growth of the rat leprosy organisms for as long as seven weeks and now provides a means for the rapid testing of possible new anti-leprosy drugs.

The bacteria, known technically as *Mycobacterium leprae murium*, are one of the most difficult types to grow outside their natural host, since they usually stop multiplying and soon die when placed in an artificial environment. Attempts by other investigators to get the organisms to grow in cell cultures have resulted in only limited multiplication, but with the new culture the number of bacteria increases seven times in seven weeks. What is even more important, however, is that during this time the organisms grow longer, and since this increase in length is known to take place before multiplication it provides proof that the bacteria are actually growing and multiplying.

Although the strain of bacteria used in the study does not cause disease in man, it belongs to the same family as that causing human leprosy. In fact, the two types are so similar that they cannot be differentiated even under the microscope, and both are related to the organism that causes tuberculosis in man. (This close similarity helps explain why a drug such as streptomycin may be effective against both tuberculosis and to some degree human leprosy or Hansen's Disease). Thus by using the new culture technique it may be possible to find a drug which is effective not only against murine (rat) leprosy but Hansen's Disease as well.

(Dr. Y. T. Chang, American Leprosy Foundation, Guest Worker, Laboratory of Pharmacology and Toxicology)

HEAT FROM ELECTRIC WIRE SPEEDS LEG BONE GROWTH

A new method for making bones grow longer by heating them with electricity has been successful in experimental animals. If the method can be applied

to humans it might provide a way not only to lengthen limbs that have been shortened by disease, but also to speed up the healing of fractures.

A shortened leg is a frequent and often very severe disability in humans. It is most commonly caused by poliomyelitis, although other conditions such as tumors and bone damage may retard bone growth. Past attempts to remedy the condition by surgically lengthening the shorter members have not been completely successful, so present-day treatment centers around temporarily retarding bone growth in the longer leg. A more ideal solution, however, would be a method of stimulating growth in the short leg, and it was for this purpose that the study of internal heating was begun.

In experimental animal work, researchers at Stanford University Medical School found that they could stimulate the growth of leg bones with heat produced by an electric wire wrapped around the bone. The wire was positioned surgically and heated with a very low alternating current. The heating, which was begun during the growth period and continued daily, raised the temperature of the bone several degrees, increased the bone's blood supply and resulted in significant increases in its length. The heating technique also caused fractures in the bone to heal more rapidly.

Certainly, the application of such a technique to humans will not be easy and before it can be attempted the preliminary studies will need to be greatly extended. However, the researchers believe that further studies are warranted since the method would be much simpler in the intelligent human patient. (Dr. Victor Richards and Raymond Stofer, DVM, Stanford University Medical School, San Francisco, California)

MOLECULAR CHANGE OCCURS WHEN MUSCLES CONTRACT

How muscle tissue converts the chemical energy of food into the mechanical energy of motion

is a puzzle that has intrigued research scientists for many years. Collaborative studies at the Institute and the National Bureau of Standards show that when muscle fibers contract, the internal structure of the long-chain protein molecules (which make up the fibers) actually change shape.

Using a technique called X-ray diffraction, investigators examined rabbit muscle fibers both in the relaxed state and in various stages of contraction. Although the fibers were non-living, they were still able to function when soaked in a chemical solution which supplied the necessary chemical energy. The degree

of contraction, seen in X-ray "scatter patterns," varied from fully relaxed to fully contracted or shortened, when the strength of the solution was changed. The patterns showed that the internal arrangement of the muscle fiber molecules changed from "ordered" to "disordered" state. Since only a slight variation in solution concentration was necessary, the action is believed to be similar in effect to the melting that takes place in some crystals at certain temperatures.

This study, the first reported to demonstrate this change of molecular shape during muscle contraction, provides important additional insight into the complex mechanism whereby living tissue converts one form of energy into another.

(Dr. Koloman Laki, Laboratory of Physical Biology, with Dr. L. Mandelkern and associates, National Bureau of Standards)

RADIOACTIVE ISOTOPE USED TO MEASURE BODY'S POTASSIUM LOSS

Surgical researchers have developed a rapid method for determining the degree of

potassium loss by the body. A depletion of this important element results in muscular weakness and can become life-threatening if the muscle of the diaphragm is affected and becomes too weak to support adequate breathing. Potassium loss often occurs in surgical patients who lose considerable amounts of gastrointestinal fluid and may become more severe when the stress response to surgery causes an increase in the urinary excretion of potassium.

The new method is valuable because it measures the store of potassium that is actually inside body cells (intracellular potassium) rather than only the amount that is circulating in blood and other body fluids (extracellular potassium). The amount of extracellular potassium can be readily measured by older methods, but this does not give a true indication of potassium loss in the tissues because the body's regulatory mechanisms will tend to maintain a normal level of extracellular potassium by drawing the needed potassium out of the cells.

The new radioactive technique rapidly measures how severe this intracellular depletion is and thus may be useful in preventing or correcting a critical condition. Essentially the method consists of adding a radioisotope of rubidium (Rb^{86}) to a blood sample and measuring the amount of the element that is taken up by the red blood cells in the sample. Because of certain similarities between rubidium and potassium, cells which are deficient in potassium will readily incorporate rubidium in its stead. Thus, the rate of rubidium incorporation indicates how

much potassium is in the cells, and an increase in the normal rate indicates potassium deficiency.

(Dr. George C. Henegar and associates, Northwestern University Medical School and the Veterans Administration Hospital, Chicago, Illinois)

NEWLY DEVELOPED TECHNIQUE MAY AID IN CYSTIC FIBROSIS

Scientists working with sialic acid--a body substance importantly involved in cystic fibrosis--

have developed a rapid and sensitive way to measure its presence in tissue. The acid, though still poorly understood, has been assuming more importance in the past decade and has been found in many body tissues and fluids. A component of mucus secretions, it is thought to be involved in the metabolic alterations that exist in cystic fibrosis, a disease in which mucus secretions become abnormally thick and sticky. The acid also has been found in increased amounts in the blood of rheumatic disease patients.

In the past sialic acid has been detected and measured by several methods, most of them relatively insensitive. The one reliable method is cumbersome and time consuming. These disadvantages have been eliminated in the new technique which is more specific and twelve times more sensitive. It promises to be especially useful in research on cystic fibrosis because studies by other investigators have shown that the amount of sialic acid present in mucus may affect its viscosity or stickiness. Also involved is fucose, a sugar present in mucus, and it appears that the relative concentrations of these two substances help determine the viscosity of the mucus. The new test will help clarify this relationship.

(Dr. Leonard Warren, Laboratory of Pharmacology and Toxicology)

SUBSTANCE RELEASED BY BLOOD CLOT MAY HELP STOP BLEEDING

Continuing research into the complex processes of blood clotting has revealed that a

substance released in the blood stream when a clot forms may aid in the control of bleeding by conditioning blood vessels to constrict.

It is known that during the last stage of blood clotting fibrin, the gelatinous material that actually forms the clot, is produced from fibrinogen in the blood by thrombin, the body's essential blood clotting enzyme. Earlier studies by scientists at the Institute showed that thrombin accomplishes this by splitting off two fragments from the larger fibrinogen molecule to leave fibrin. The resulting two molecular fragments, composed of long chains of amino acids, were named peptide A and peptide B.

Further studies on peptide B have now revealed that in low concentrations it can "sensitize" or condition the smooth muscle inside the walls of arteries in such a way that the muscle responds to an electrical stimulus much more strongly than it otherwise would. Although the work was done in isolated arterial tissue from a rabbit, the finding suggests that peptide B may play an important role in the control of bleeding. Presumably, the peptide would be released during the clotting process and by speeding the closure of damaged blood vessels would provide for the firm fixation of the clot. Further studies of the phenomenon will be needed, using human material, but if they provide similar results the research may have important implications not only in the blood clotting process but also perhaps in understanding conditions that lead to hypertension.

(Drs. Koloman Laki and Jules A. Gladner, Laboratory of Physical Biology, and Dr. J. E. Folk, National Institute of Dental Research)

CASE OF A PATIENT SENSITIVE TO HER OWN BLOOD IS REPORTED

The rare case of a woman sensitive to her own red blood cells has been reported by scientists studying the causes of various blood disorders. Only five other cases of this disorder--autoerythrocyte sensitization--have been reported in medical literature. In each case, slight bleeding into the skin was followed by large, painful bruises which occurred without apparent trauma. The painful bruises could also be produced by injecting minute amounts of red blood cells into their skin.

In the newly-discovered case the patient was not only more sensitive to injected red blood cells taken from her own blood stream than the previously reported cases, but she also suffered marked weakness, nausea and flushing in association with the bruises.

In the study, the investigators found that injected histamine, or histamine-releasing substances, were able to produce the same reactions in the skin of the patient, although neither caused a reaction in the skin of normal persons. This finding, which suggests that histamine acts as a mediator of the reaction, is providing the basis for further studies into the cause and treatment of this disease and others of suspected tissue sensitivity.

(Dr. N. Raphael Shulman and associates, Metabolic Diseases Branch)

STUDIES OF GAMMA GLOBULINS REVEAL THEIR IMPORTANCE

The recent and varied roles played by the gamma globulins-- a group of antibody-containing

proteins present in blood--in preventing or combating various diseases was pointed up in a significant review report prepared by a group of Institute-supported scientists. In addition to the protective function of the gamma globulins they are known to play a major part in diagnostic tests for rheumatoid arthritis. (The rheumatoid factor found in the blood of patients with this disease causes the agglutination or clumping together of gamma globulin or particles coated with gamma globulin.)

Pooled and concentrated gamma globulin derived from normal healthy blood donors contains enough disease-protecting antibodies to either completely prevent or help overcome such diseases as measles, infectious hepatitis, German measles and poliomyelitis. Protection against infectious hepatitis can be achieved with a dose as low as 0.01 milliliters per pound of body weight if given within 14 days after exposure. In poliomyelitis, gamma globulin treatment apparently has a modifying effect on the paralytic complications if given during the first five to seven days of the incubation period. In measles, complete prevention can be attained in 80 percent of intimately exposed, susceptible children by small doses of gamma globulin given during the first six days after exposure. (Dr. David Gitlin and associates, Harvard Medical School and Children's Hospital, Boston, Massachusetts.)

PROGRAM DEVELOPMENTS

INTERNATIONAL MEETING ON "ARTHRITIS" PRODUCING AGENT

The first international meeting on pleuropneumonia-like organisms which produce a disease resem-

bling arthritis in animals, was held in January, 1959, under the auspices of the New York Academy of Sciences. Two hundred and twenty scientists from ten countries attended the conference. Included were representatives from the National Institute of Arthritis and Metabolic Diseases, the Division of Biologic Standards, and the National Institute of Allergy and Infectious Diseases, which sponsored the conference.

Relatively few studies of the pleuropneumonia-like organisms (PPLO) have been made since 1898 when the Pasteur Institute first found the microorganisms in animals, but there is now a growing concern about the significance of these agents. In many ways PPLO resemble certain forms of bacteria, which also grow without a rigid cell wall, but the fact that they pass through many bacterial filters has inclined some investigators to class them with viruses. The organisms continue to pose a threat to livestock and poultry producers, and entire herds of cattle have been destroyed in order to eradicate diseases from an area. Following the first isolation of PPLO from humans in 1937, the organisms were found to be more widely disseminated and more closely associated with disease than had previously been recognized.

PPLO organisms have been recovered from the human intestinal tract, the fluid of arthritic joints and from eye tissue and skin lesions. Because injections of the material into rats, mice and other experimental animals has produced a condition somewhat similar to arthritis in man, scientists are trying to determine if these agents play some causative role in human arthritis. The conference served to integrate information from various scientific disciplines on the cultural requirements, morphology, biochemistry, serology and pathology of PPLO, and included discussions of a number of species of this agent. In the past, controversies over the place of PPLO in microbiology and in the etiology of disease have prevented the development of a concerted and cooperative research attack. This first international meeting served to bring the problem into sharper focus, re-emphasize its importance, and point out fruitful areas for further research.

U. S. NUTRITION SURVEY TEAMS EXPAND FOREIGN OPERATIONS

The nutrition survey program of the Interdepartmental Committee on Nutrition for National Defense (ICNND) is now being expanded to include large samplings of civilian as well as military populations. This program was started three years ago to aid the developing countries of the world in improving the nutritional health of their people, and surveys have now been completed in 12 foreign countries.

The ICNND, which operates administratively through the National Institute of Arthritis and Metabolic Diseases, was established in 1956 as part of the U. S. Mutual Assistance Program. One of its major activities is sponsoring nutrition survey teams which spend approximately 30 days in each country, studying food and nutrition problems and establishing a nutrition service which will continue to function after the team departs. Each team consists of physicians, biochemists, nutritionists, food specialists, statisticians and dental researchers who travel throughout the country examining large samples of the population. The sampling of civilian populations in addition to the military began with the ninth ICNND survey, made in Ethiopia. This has enabled the teams to make a more accurate appraisal of the overall nutritional health of the people.

One of the most serious problems in underdeveloped countries is the lack of sufficient food, especially food containing high quality protein. In countries where rice is the main source of protein, the rice is usually polished at the mills to enhance its keeping qualities, but unfortunately this polishing process removes valuable vitamins and mineral elements. One example of the positive results of the nutrition surveys are two new mills for rice enrichment which were built in the United States and shipped to Formosa. The mills make possible vitamin-mineral coating of the rice at extremely low cost and can provide the people of Formosa with adequate dietary intakes of thiamine, riboflavin, niacin and iron.

ICNND nutrition surveys have now been completed in Korea, Iran, Pakistan, the Philippines, Turkey, Libya, Alaska, Spain, Ethiopia, Peru, Ecuador and Viet Nam. A survey is planned for Chile in April, 1960.

NIAMD IS HOST TO PAN AMERICAN RHEUMATIC DISEASES CONGRESS

More than 600 rheumatologists and research scientists from 15 countries throughout North and South America attended the Second Pan American Congress on

Rheumatic Diseases held in Bethesda, Md., and Washington, D. C., June 2-6, 1959. The Institute was host for two days of scientific sessions held in the NIH Clinical Center auditorium. A highlight of these sessions was the presentation of a "live clinic" demonstration via the Clinical Center's closed circuit television system. The meeting, under the auspices of the Pan American League Against Rheumatism, was organized by the American Rheumatism Association and was held in conjunction with that organization's 23rd annual meeting. The Institute contributed substantially to the support of the Congress, as did the Arthritis and Rheumatism Foundation.

Long-term trials of dexamethasone, one of the newer antirheumatic steroid drugs, were reported by several groups of investigators. Also reported at the Congress were experiences with a new uricosuric (uric acid-removing) drug for the treatment of gout.

Institute scientists reported to the Congress that arthritis has been found to occur among the Alaskan Eskimos. The Eskimo study showed that both rheumatoid arthritis and osteoarthritis exist in this far northern area, but accurate prevalence figures were difficult to compute since very few Eskimos live past middle age. A South American survey of arthritis in Chile was also reported to the Congress by an investigator from Chile, who found that the incidence of both rheumatoid arthritis and osteoarthritis has increased since 1925. A decrease in the incidence of rheumatic fever has taken place during the same period in Chile.

INSTITUTE DEMONSTRATIONS AID SCIENCE TEACHING IN SCHOOLS

More than 200 high school science teachers from the District of Columbia, Maryland, Virginia and Delaware attended a one-day science demonstration conference co-sponsored by the National Institute of Arthritis and Metabolic Diseases and the National Cancer Institute, in October, 1959. The meeting was held in collaboration with the U. S. Office of Education and the National Science Teachers Association to help emphasize the increasing importance of a high level of science teaching in the school, and to provide teachers with an opportunity to broaden their science background.

The teachers, in small groups, visited laboratories, met scientists and saw presentations of research projects, techniques and instrumentation. In the afternoon, teachers visiting the National Institute of Arthritis and Metabolic Diseases saw a closed-circuit television presentation of that medium as an educational tool. During the one and one-half hour TV presentation, both laboratory and clinical demonstrations of research in gout, alcaptonuria, the motility of leucocytes and capillary circulation were shown.

The wholehearted endorsement and spirited cooperation of Institute scientists, who devoted their own time to the demonstrations,

was matched by the enthusiasm of the attending teachers. A survey showed that almost all the teachers rated the program "excellent," believed that they could make direct application, in the classroom, of much of the information presented to them, and hoped that the program could be repeated at regular intervals. Because of the success achieved, similar programs may be presented periodically in the future.

HUMAN AND ANIMAL FORMS OF RHEUMATIC DISEASE COMPARED

The first conference specifically designed to discuss the comparative (animal vs. man) pathology of arthritis and rheumatism was held in Washington, D. C., in February, 1959, under the sponsorship of the National Institute of Arthritis and Metabolic Diseases, the American Rheumatism Association and the Arthritis and Rheumatism Foundation. The two-day conference was attended by 45 specialists including investigators from Sweden and Denmark.

One of the problems in arthritis research has been the lack of experimental animals which develop rheumatoid arthritis. The disease does not occur naturally in animals, and attempts to induce it have not been successful. Because of this, a great deal of interest has centered around animal diseases which are analogous in some respects to the arthritic and other rheumatic diseases of humans. Several such diseases were reported at the conference. One type is a transmissible joint inflammation which attacks poultry and livestock. Two filterable agents (viruses and mycoplasmae) have been recovered from infected animals and are now known to cause the animal disease, but to what extent these specific agents may be involved in human inflammatory joint disease is speculative.

Osteoarthritis, congenital hip disease, slipped discs and hemophilic joint disease all are fundamentally similar in both animals and man. These conditions, discussed at the conference, are common in animals, providing readily accessible material for the study and control of such degenerative disorders. Gout, one of the rheumatic diseases affecting man, also occurs in birds, and causes some of the same tissue reactions. The birds develop tophaceous deposits of uric acid similar to those found in man, although the metabolic mechanisms involved are considerably different.

The conference resulted in a much clearer definition of the fundamental similarities and dissimilarities between human and animal arthritis. The proceedings are being edited and will be made available in a separate volume to interested veterinarians, pathologists and clinicians. They have also been published as a special supplement to the journal Laboratory Investigation.

HIGHLIGHTS OF PROGRESS

IN DENTAL RESEARCH

1959

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Institute of Dental Research

HISTOLOGY AND PATHOLOGY

DENTAL CARIES RECOGNIZED AS INFECTIOUS AND TRANS- MISSIBLE DISEASE

Dr. Paul H. Keyes, NIDR Laboratory
of Histology and Pathology, reported
this year on studies which show
dental caries in laboratory

animals to be an infectious and transmissible disease involving a
penicillin-sensitive flora.

The caries resistance and susceptibility phenomena in conventional laboratory animals has until recently been attributed primarily to genetic factors and to systemic developmental effects presumably induced by diet and nutrition. In a series of studies on the nature of dental caries in hamsters and rats, Dr. Keyes has demonstrated that the penicillin-sensitive bacterial flora may be of equal or greater pathologic significance in the induction of this disease.

It now appears that the source of the caries producing microbial flora in young animals is from the alimentary tract of the mother; and that animals lacking this flora may acquire it by cross infection contact with caries active animals. However, while the cariogenic flora can be transmitted between members of the same strains, it is not ubiquitous in the general laboratory environment and may, in fact, require considerable time to become established at pathogenic levels. Work now in progress suggests that there may be limitations either to the extent the flora of one species can be transmitted to another, or to the degree it will be pathogenic if transmitted.

This observation may explain, in part, why previous attempts to induce caries in laboratory animals by the inoculation of non-indigenous strains of bacteria have failed.

Findings from this series of important studies have already had a widespread significance in the field of experimental caries research and are providing a firmer basis for more definitive studies of factors influencing dental caries.

POTENTIAL DIAGNOSTIC
PROCEDURE FOR AMYOTROPHIC
LATERAL SCLEROSIS

Dr. H. M. Fullmer, NIDR Laboratory
of Histology and Pathology, in
collaboration with Doctors, L. T.
Kurland, H. D. Siedler, and R. S.

Krooth of the National Institute of Neurological Diseases and Blindness, has demonstrated through histochemical procedures a heretofore unobserved change in connective tissue of skin from patients with amyotrophic lateral sclerosis.

During the course of histochemical studies of connective tissues, investigators in the NIDR and NINDB observed that abdominal skin taken from patients with amyotrophic lateral sclerosis stained much more densely when treated with the peracetic acid-aldehyde fuchsin-Halmi stain than did skin taken from control patients. Further study of the specially stained tissues showed a definite alteration of collagen bundles; a marked tendency for elastosis; increased amounts of mucopolysaccharides in the dermis; and, degeneration of arrector pili muscles, also associated with increased amounts of mucopolysaccharides. Analyses of 39 cases and an equal number of controls, have not shown the observed changes to be related to the age or sex of the patient or to the duration or severity of the disease. Additional data bearing on this heretofore unobserved phenomenon will be necessary to determine if the skin changes seen in ALS patients are associated with a primary metabolic defect, or are secondary to the loss of innervating neuronal elements.

Studies measuring the specificity of tissue changes in amyotrophic lateral sclerosis as contrasted with other degenerative neurological or myopathic diseases are now being conducted. In addition to skin, organ tissues will be studied to determine whether abnormalities in mucopolysaccharides concentrations can be detected histochemically. If these and related investigations lead to the conclusion that the observed changes do indeed reflect a primary metabolic abnormality, then a reliable and specific histochemical method will have been developed for the early detection of amyotrophic lateral sclerosis.

METABOLIC DISTURBANCES IN TOOTH FORMATION

Despite advances in the understanding of the etiology and pathogenesis of abnormal tooth development, clinical and experimental data are almost wholly confined to the postnatal period, and only limited information is available today on the subject of prenatal influences. Studies designed to better elucidate the relationships of various metabolic and viral agents to congenital anomalies of the teeth were discussed at a conference of the New York Academy of Sciences this year.

Speaking at the Conference on Metabolism of Oral Tissues, Dr. Seymour J. Kreshover, Associate Director, NIDR, reported that while defects in tooth development are generally thought of as postnatal in occurrence, there is an increasing awareness of their pertinence to the broad field of congenital anomalies. In this area, the prenatally occurring dental defects may be not only indicative of a widespread fetal response to adverse maternal influences, but may also provide a method for evaluating the nature and mode of maternal-fetal disturbances.

It is known that many types of prenatally and postnatally occurring systemic disturbances, including fever and anoxia, may elicit a similar ameloblastic response. For example, in a study of the effects of pyrexia on the course of pregnancy in rats, striking changes occurred in the developing dentitions of the mother. These ranged from early ameloblastic injury to complete cellular degeneration and arrest of enamel matrix formation. Identical enamel defects also occurred in the offspring of pyrexemic rats. In the case of such metabolic disturbance as alloxan induced diabetes, pregnant rats and their young showed similar dental abnormalities.

Relative to the question of transplacental passage of viral agents, striking abnormalities were observed in the developing teeth of female rabbits inoculated with measured doses of an egg adapted dermal lymph strain of vaccinia virus. A failure to demonstrate any congenital dental defects in caesarean delivered progeny of these rabbits affords an interesting parallel to the observation that vaccinia infection does not cross the placental barrier.

Dr. Kreshover concluded by suggesting that developmental tooth defects are generally nonspecific in nature and can be related to a wide range of systemic disturbances any of which, depending upon their severity and the degree of tissue response, might result in defective enamel and dentin.

ELECTRON MICROSCOPE STUDY OF MOTTLED ENAMEL

The causal relationship of excess fluorides to dental fluorosis (mottled enamel) has long been

established. The mechanism by which this abnormal condition occurs is being studied by NIDR scientists using the electron microscope to observe structural differences in surface and subsurface enamel.

Previous studies by the NIDR and others have provided considerable information on the microsurface details of non-fluorosed enamel. In recent studies by Drs. D. B. Scott, Laboratory of Histology and Pathology, and P. O. Pedersen, Royal Dental College, Copenhagen, Denmark, microscopic examinations were made of the enamel surfaces of 485 teeth in situ, and 146 extracted teeth, all showing evidence of chronic endemic fluorosis. Metal shadowed collodion replicas of over 800 surfaces from these teeth were first examined under the optical microscope. From these, 100 were selected and shadowed carbon replicas were prepared for electron microscopy study.

Data gathered from electron microscopic observations of the white opaque and chalky regions, as well as pitted areas, all characteristics of dental fluorosis, indicated that the constitutional differences between fluorosed and normal enamel are extremely subtle. This was strikingly demonstrated by the fact that, except in the case of pits, differences are revealed only after the microscopic wearing away of the surface structure (part of the aging process) has begun. As indicated by selective damage to the surface, it appears that the areas which show the gross signs of fluorosis are actually slightly less resistant to the external wearing process.

Further exploration of the characteristics of these weaker regions through the experimental application of various chemical and physical forces promises greater insight into the suspected variance in organic and inorganic components of affected enamel.

OSTEOGENIC POTENTIAL OF CALCIUM HYDROXIDE

The search for a truly osteogenic substance has been in progress for many years. In the last several

decades numerous chemical compounds, extracts, vaccines and sera have been used to stimulate bone growth without success. NIDR grantees at Indiana University report this year on the significant osteogenic potential demonstrated by calcium hydroxide when implanted in connective tissue of animals.

In a study of tissue reaction to various dental materials, Drs. D. F. Mitchell and G. B. Shankwalter implanted small pellets of calcium hydroxide (2 x 2 mm) in 17 adult, male white rats. Two skin incisions were made on the backs of each of the anesthetized animals where the pellets were placed subcutaneously. Wounds were then closed with sutures. One animal was sacrificed at each of 17 intervals from 2-87 days and the tissue surrounding and including the implant was excised, fixed, mounted and stained, for microscopic examination.

In observations of sectioned tissue a form of heterotropic ossification or calcification was consistently found in the soft tissue surrounding the specimens of calcium hydroxide studied at 13 of 17 selected intervals. The earliest appearance was 10 days with bone-like material appearing during a 10-35 day period. In some cases the calcified material closely resembled bone. In other specimens it appeared that muscle bundles were partially calcified resulting from contact with the calcium hydroxide.

Evaluation of compounds that may show osteogenic potential by screening through implantation in connective tissue may lead to the discovery of clinically useful agents for the activation or acceleration of bone healing. Such compounds would find wide application not only in the field of dental surgery but in the whole field of orthopedic medicine.

IMPROVED TENDON PRESERVATION TECHNIQUE FOR ELECTRON MICROSCOPY

Dental investigators have reported the development of improved histological methods that more nearly preserve the true structure

of collagen fibrils than heretofore achieved in sectioned material.

In investigations of the relationship between collagen fibrils and mineral crystallites at the electron microscopic level, the preservation of tissue is of vital importance. Almost all of the information, to date, on the fine structure of biological tissues as revealed through electron microscopy has been gained from sections of material fixed with buffered osmium tetroxide dehydrated in alcohol, and embedded in plastics.

During studies of sections of the leg tendon of the domestic turkey, which is being used as a "model system" in basic studies on bone and tooth calcification, Drs. David B. Scott and Marie U. Nylen, NIDR Laboratory of Histology and Pathology, observed that extracellular structures frequently suffered considerable damage when processed by routine methods. This was particularly true in the case of the collagenous component, where the microfibrils appear to become unduly separated.

To determine if this structural pattern does, indeed, represent a true picture of the fibrillar organization of intact tendon, the investigators subjected specimens to different preparatory methods for comparison. They found that when acetone and ethyl chloride was substituted for the osmium tetroxide fixation and alcoholic dehydration steps, or for the fixation step alone, the tendon fibers presented a markedly different appearance under the electron microscope. The collagen microfibrils were in close contact and formed wide bands with definite striation alignment. Furthermore, the sheet-like configuration resembled that seen in preparations of both teased native collagen fibrils and reconstituted collagen.

While better methods for preserving collagenous fiber patterns more nearly like they exist in vivo is of inestimable importance to the present study of calcified structures, such improved techniques will undoubtedly find wider application in studies of connective tissues in general.

NEW HISTOCHEMICAL TECHNIQUES

Dr. M. S. Burstone, NIDR, Laboratory of Histology and Pathology, has reported the development and application of new histochemical methods for the demonstration of cytochrome oxidase, the major respiratory enzyme.

Recognizing that various reagents that have been used to histochemically demonstrate cytochrome oxidase possess a basic instability and susceptibility to auto-oxidation, Dr. Burstone has devised techniques which facilitate much finer localization of the cytochrome oxidase in microscopic sections than has hitherto been possible; as for example, its microscopic demonstration in mitochondria. With this new procedure stained slides are permanent and resultant dyes are capable of complexing with heavy metal, thus demonstrating a potential application in electron microscopy. The formation of metal complexes is additionally significant as the intensity of the final dyestuff is measurably enhanced thereby permitting a substantial-ly shorter incubation period.

Current applications of the described technique are being explored with Dr. G. G. Glenner, National Institute of Arthritis and Metabolic Diseases, in connection with mitochondrial localization of tissue oxidase in human myocardial infarctions. This may offer new information on incipient metabolic changes in coronary disease.

STIMULUS MECHANISM IN THE FORMATION OF PERIODONTAL TISSUES IS STUDIED

For his significant studies on formation of periodontal tissues, Dr. Richard L. Hoffman, NIDR Research Fellow at the University of Illinois, was recipient this

year of the Hatton Award presented by the International Association for Dental Research.

To determine whether the stimulus for periodontium formation is located within or outside the developing enamel organ and dental papilla, Dr. Hoffman transplanted hamster molar tooth germs into the subcutaneous tissue of host animals. Transplanted tissue was from 13-, 14-, and 15-day in utero fetuses, and 5-day-old donors in which no periodontium had formed.

From a total of 112 transplants, 59 were recovered after 28 days of growth and development in the host. Of these, 72% showed formation of periodontal ligament and alveolar bone in the tissue organized around their roots. These findings suggest that the stimulus for periodontium formation resides in the developing tooth and may be associated with the enamel epithelium and proliferating epithelium of the root sheaths.

Of special interest was the observation that even though the transplanted teeth had developed entirely within the loose connective tissues under the skin, the fiber orientation of their periodontal ligaments was found to be similar to normal teeth which had developed and functioned in situ. These observations thus opened for reconsideration the influence of stress of mastication on the morphology of dental organs.

BLOOD FLOW TO TEETH INFLUENCES CARIES

NIDR grantees at Indiana University reported on the relationship between decreased vascularity to teeth and the dental caries experience in animals.

Since the vascularity of organs is important for proper physiologic maintenance, Drs. H. E. Brewer and J. C. Muhler, Indiana University undertook a study to correlate the possible increase in dental caries experience in rats with a decrease in blood supply to the molar teeth.

A total of 120 weanling rats were divided equally into two groups according to sex and initial body weight. The principal arteries supplying the teeth of animals in the experimental group (group 1) were ligated bilaterally, while group 2 animals were not operated and served as controls. Both groups were then maintained on a cariogenic diet. At the termination of the experimental period of 104 days all animals were sacrificed. Mandibles of control and experimental animals were sectioned for dental caries evaluation.

Data collected showed a 43% increase in dental caries experience in those animals deprived of normal blood flow. Further, the mean number of molars affected, as well as the severity of the lesions, indicated a higher incidence of dental caries in those animals subjected to surgical procedure.

Although the reasons for the increased caries susceptibility in animals with decreased blood supply is not completely understood, notable change in the physiology of the hard and soft dental tissue was observed and appears to be an implicating factor in the caries process.

MICROBIOLOGY

DENTAL INVESTIGATORS
REPORT ON VINCENT'S
INFECTION

Dr. E. G. Hampp, American Dental Association, Senior Research Associate at NIDR, speaking before the Society of American Bacteriologists

this year reported on research studies which may further elucidate the interrelationship of oral spirochetes and necrotizing ulcero-membranous gingivitis (Vincent's Infection).

For many years the presence of oral spirochetes has been closely associated with Vincent's Infection; however, it was not until 1944 that Borrelia vincentii, the organism thought to be responsible for Vincent's Infection, was first isolated and cultured on artificial medium by Dr. Hampp. In subsequent years all known morphological types of spirochetes including B. Buccalis were isolated and characterized. Numerous but unsuccessful attempts were made by dental investigators to produce experimental infections in a variety of laboratory animals, utilizing different sites of inoculation with various pure strains of spirochetes, both singularly and in combination.

Recently, Dr. Hampp and Dr. S. E. Mergenhagen, Laboratory of Microbiology, found that intracutaneous inoculation of rabbits with concentrated pure individual cultures of B. vincentii and B. buccalis resulted in lesions that terminated in abscesses in approximately four days. Spirochete organisms were successfully cultured from the skin abscesses and when reintroduced into the skin of other animals, similar lesions were produced. Evidence of the toxicity of the spirochetes was demonstrated by the production of characteristic lesions and localized necrotic tissue in animals inoculated with heat-killed organisms. No lesions were produced from inoculations of supernatant broth of heat-treated and non heat-treated cultures.

Results of this study provide the first positive evidence that reproducible lesions can be initiated with pure cultures of various strains of oral spirochetes. These findings are attributed, in part, to the selection of the site of inoculation and the use of large numbers of organisms in the infective dose. Further investigation of the mechanism of this infection may confirm the long suspected pathogenic significance of oral spirochetes in soft-tissue lesions of the mouth

PARASITIC PROTOZOA
IMPLICATED IN
DENTAL DISEASE

Parasitic protozoan inhabitants of the mouth first received attention in 1915 when investigators incriminated Endamoeba gingivalis

as a probable cause of periodontal disease. Since that time subsequent investigations have failed to confirm or disprove these earlier reports.

NIDR grantee, Dr. Wayne W. Wantland at Illinois Wesleyan University has reported on studies of two recognized oral parasitic protozoa and their possible relationship to dental diseases. In observations of 80 patients ranging from 6-60 years of age, the incidence of Endamoeba gingivalis and Trichomonas tenax was 40% and 22% respectively, while 18% of the patients harbored both parasites. 20% of patients examined had neither organism. E. gingivalis was found most frequently in persons 41-50 years; T. tenax most frequently in persons 31-40 years. E. gingivalis was detected in each of 7 cases of periodontal disease, 4 of which also showed T. tenax. In patients showing good oral hygiene classified as clean normal, 13 harbored E. gingivalis, 5 harbored T. tenax and 4 carried both protozoans. Persons harboring oral protozoa particularly E. gingivalis, showed a greater number of carious teeth than persons free of these organisms.

While both parasites are prevalent in "healthy mouths," they have been found especially prevalent in dental caries, pyorrhea and other suppurative and inflammatory conditions of the mouth and throat. Although there is virtually little data at present to support a causal relationship of these protozoa and oral disease, there is evidence that suggest these scavenger parasites play a significant role in providing optimum environmental conditions for the propagation of other oral microbial flora.

BIOCHEMISTRY

COLLABORATIVE STUDIES OF BLOOD CLOT MECHANISMS

A great deal of fundamental biochemical research has been devoted to attempts to explain the clotting of blood, and many theories have been proposed. All, however, have been somewhat insecure in identifying the action of thrombin on fibrinogen as many of the substances involved have gone unrecognized or have been incompletely understood. Investigators in the Institutes of Dental Research and Arthritis and Metabolic Diseases reported on collaborative studies that further elucidate the mode of action of thrombin on fibrinogen.

The clotting of the soluble protein, fibrinogen, is the last in a series of steps in the process of blood coagulation. In these steps the enzyme thrombin acts on fibrinogen resulting in the formation of a fibrin-gel. This gel plugs the wound and thus stops the flow of blood.

Previous studies, as well as current investigations by Dr. J. E. Folk, Laboratory of Biochemistry, NIDR, and Drs. K. Laki and J. A. Gladner, National Institute of Arthritis and Metabolic Diseases, demonstrated

that the thrombin catalyzed clot formation occurs as an enzymatic reaction followed by polymerization. The conversion of fibrinogen to fibrin by thrombin, which may be seen as formation of crystalline structures, is preceded by the release of a nonprotein nitrogen material consisting primarily of the two acidic peptides, A and B. Investigations have further shown that this rupture or splitting of the fibrinogen molecule by thrombin is specific and probably occurs by the hydrolyzation of certain arginine-glycine bonds in the N-terminal vicinity of the fibrinogen molecule.

Current studies by NIDR investigators on the amino acid sequence of peptides A and B have contributed to a better understanding of the factor or factors of substrate structure that relate to the specificity of thrombin, as well as defining the precise manner by which fibrinogen is prepared for polymerization through release of these peptides. Complete answers to these and other questions will, while contributing to a better understanding of the clotting mechanism, offer greater insight into the complex enzymatic reactions implicated in the breakdown of oral tissues as seen in periodontal disease.

GRANTEES DEMONSTRATE LYMPHATIC DRAINAGE OF TEETH

Previous studies on the circulation of lymph in dentin and enamel in dogs have shown that there is a flow of lymph along the tubules of

dentin and that this irrigation by the body fluids takes place in the tubules in either direction according to varying pressure in the surrounding tissue. NIDR grantees have demonstrated this year through the use of radioactive colloidal gold, the occurrence of lymphatic drainage from the tooth by the lymph nodes.

In a study designed to clearly demonstrate this drainage from canine teeth, L. Kraintz, C. Tyler and B. Ellis of the University of Texas, placed radioactive colloidal gold (Au^{198}) in a cavity preparation extending into the dentin of a lower cuspid tooth. The preparation was then sealed with a low fusing wax. The radiogold was allowed to stay in the tooth for four hours. At the end of that time the left and right submandibular lymph nodes were removed along with the cervical and inguinal lymph nodes. Assay for radioactivity was accomplished by scintillation counter.

Data obtained showed detectable radioactivity four to five times above background in the submandibular lymph node adjacent to the experimental tooth. No radioactivity was detected in the other lymph nodes, indicating colloidal gold did not enter the general lymphatic circulation in significant amounts.

Identical experiments were repeated on 8 dogs with positive results occurring in 6 of the animals. Physical occlusion in the dentinal tubules during cavity preparation and the age of the dogs were factors presumed responsible for negative results.

CLINICAL INVESTIGATIONS

ENZYME ACTIVITIES
ASSOCIATED WITH
CALCULUS FORMATIONS

The manifold nature and great variety of factors associated with periodontal disease presents an elusive dental research problem

today. While laboratory and clinical studies have identified calcified deposits on teeth as a pathologic factor contributing to periodontal disease, many questions concerning the mechanism of calculus formation remain unanswered. NIDR scientists reported this year on the use of enzyme histochemistry as a tool for the study of early calculus deposits.

In combined laboratory and clinical studies carried out by Dr. Paul N. Baer, Clinical Investigations Branch, and Dr. M. S. Burstone, Laboratory of Histology and Pathology, soft deposits, identified as early calculus, were formed on Mylar strips placed in the mouths of periodontal patients. Histologic examination of strips incubated in substrate solutions to show presence of esterase activity revealed the bulk of the deposits to be clumps of coccoid and filamentous forms of bacteria. Esterase activity was discernible as an intense red stain in both microorganisms, with cocci showing highest activity. Diminishing esterase activity of leukocytes, macrophages, and epithelial cells was also evident and appeared to be correlated with cell vitality.

One recognized concept of calcification holds that fatty acid esters represent a determining factor in pathologic calcifications by means of a saponification mechanism. In this regard, it is noted that hydrolysis of fatty acid esters by enzyme (esterase) activity may release fatty acids which are presumably capable of forming calcium and magnesium soaps. These soaps, which have the appearance and consistency of early deposit formations, may then undergo transformation into the less soluble phosphate and carbonate, both recognized as constituents of oral calculus. Further study of this mechanism, may provide confirmatory evidence that esterase activity in tooth deposits is correlated with a saponification process which ultimately leads to calcification.

GENERAL ANESTHESIOLOGY
IN AMBULATORY DENTAL
PATIENTS

Today, in many geographic areas it is estimated that almost as many people are given general anesthesia in dental offices as in hospitals.

These anesthetics are performed on outpatients and differ from hospital surgery procedures in that the patients are ambulatory, often unpremedicated, nervous, and expect to be incapacitated for short

periods of time only. Furthermore, surgery is performed in the oral cavity, a part of the vital airway where blood, saliva and operative debris are potential hazards to the patency of the airway.

A clinical study designed to gather base line physiological data related to the use of general anesthesia in oral surgical procedures was the subject of an NIDR exhibit at the Centennial Meeting of the American Dental Association this year. The exhibit depicted a series of studies by Dr. E. J. Driscoll, Clinical Investigations Branch, (assisted by Dr. G. R. Christenson, Assistant Chief, Anesthesiology Department, Clinical Center, and Mr. C. L. White, Epidemiology and Biometry Branch), and relates to the physiologic effect of anesthetic agents currently being used in oral surgery. Data on patient reaction to intravenous barbiturates, used alone and in combination with such other anesthetic agents as nitrous oxide, establishes base lines of physiologic performances for comparison with the newer drugs being synthesized today. Clinical records on patients subjected to oral surgical procedures were obtained for evaluation through continuous pulse, electroencephalographic and electrocardiographic monitoring.

Inasmuch as dental anesthesiology calls for special techniques, skills and agents, data gathered from this study are expected to find increasing application as new and more promising drugs are made available to the oral surgeon.

DENTAL-PSYCHOSOMATIC PROGRAM

In recent years psychosomatic relationships in the human body have been the subject of much discussion

and investigation. Yet, in one significant area--the oral cavity--these relationships have been largely ignored, despite a growing quantity of statistically evaluated data substantiating the fact that many oral pathologic conditions may have psychological bases. NIDR grantees at Boston University are engaged in studies designed to show, through laboratory experimentation, that dental caries and/or periodontal disease may be produced by emotional stimuli or tensions.

Investigations, to date, of the interrelationship of oral diseases and psychologic stress have been based almost wholly on clinical or informal observations and impressions. During the period beginning with World War II an increasing number of writers in dental research, as well as in psycho-pathology, reported on the significance of anxiety or emotional stress as a factor in dental and oral symptoms. For example, it is a generally accepted clinical impression that during high periods of tension the gastric secretions may change the chemical equilibrium of the mouth and contribute to the alteration of pH and viscosity of the saliva thus rendering the teeth more susceptible to caries and contributing to a change in the gingival tissue. Such observations are making it increasingly clear to both medical and dental clinicians that emotional factors do indeed play a significant role in the precipitation or aggravation of dental disturbances. Other

reports, related to the apparent acceleration of oral disease in our armed forces during periods of combat stress in World War II and the Korean conflict, have contributed further documentation to the subject of psychosomatic factors in dentistry. At the present time, Navy dentists are trying to determine why men serving on submarine duty suffer dental decay twice as fast as other Navy personnel. Similar problems are currently being anticipated as man is readying for space travel.

In recognition of the increasing need for experimental data as well as confirmation of existing clinical findings, the National Institute of Dental Research recently activated an investigative program to study the effects of experimentally induced psychologic stress on dental tissue. These studies, under the direction of Dr. Leo J. Reyna, NIDR grantee in the Psychology Laboratories at Boston University, are designed to identify and demonstrate, through control experiments with laboratory animals, the suspected role of psychologic stress as a causal factor in oral disease. A more advanced program of research to evaluate factors of stress is now under way in the Clinical Investigations Branch of the Dental Institute. This large-scale clinical study, in collaboration with the University of Pennsylvania School of Dentistry, is concerned with the pathogenesis of aphthous stomatitis, a painful and disabling oral condition usually associated with emotional stress. These studies are expected to provide important confirmation of the still empirical clinical impressions that relate to psychosomatic factors in oral disease.

HERPES VIRUS AND APHTHOUS STOMATITIS INVESTIGATED

Herpetic lesions occur in two principal forms--herpes labialis appearing on the vermillion border of the lip, and acute herpetic

gingivostomatitis appearing on the intra-oral mucosal and gingival tissues. Recent studies by NIDR investigators, in collaboration with other National Institutes of Health scientists, have provided substantiating evidence that a third disease entity (termed aphthous stomatitis) which occurs intra-orally and closely resembles the herpetic lesion, is probably of nonviral origin.

At a Combined Clinical Staff Meeting this year representatives of NIDR, the National Institute of Arthritis and Metabolic Diseases, and the Division of Biologics Standards reported on findings differentiating the above mentioned lesions. Viral studies were outlined by Drs. S. Baron, DBS, J. P. Utz of NIAID, and I. I. Ship of the NIDR Clinical Investigations Branch, in which all attempts to isolate the herpes simplex or other virus from aphthous stomatitis lesions were unsuccessful. Further differentiation between the

viral and nonviral lesions was demonstrated by Dr. H. R. Stanley, Jr., of the NIDR Clinical Investigations Branch in a histopathologic study which showed that aphthous stomatitis does not exhibit the clearly discernible vesicular stage characteristic of the herpetic lesions. In addition, the aphthous stomatitis lesions extend more deeply into the tissues, and often persist for longer periods.

Adding further to new knowledge of the etiology and pathogenesis of the herpetic and nonherpetic diseases has been an epidemiological study of a large group of college students in the Philadelphia area. Data from these investigations will complement the earlier clinical-pathological studies, and thereby provide an increasingly broad base of fundamental information on the etiological differences between the two diseases.

HAZARDS OF ADMINISTERING ANESTHETICS IDENTIFIED

In the so-called "healthy mouth," saliva contains about 750 million microorganisms per cc, from which approximately 50 species have been isolated to date. Dental grantees at the University of Miami this year reported that microbial infection may occur during the procedures attending routine injection of local dental anesthetics.

In clinical studies of these hazards, Drs. M. M. Streifeld and D. D. Zinner have demonstrated an involuntary aspiration of microorganisms into hypodermic needles and anesthetic cartridges commonly used in dental practice today. Involuntary bacterial aspiration into hypodermic needles and cartridges after oral injection through non-medicated mucosae of 274 patients resulted in a significantly high incidence (34% and 55%) of gross contamination of both instruments. Preparation of the site by wiping away saliva and applying a surface antiseptic (tincture of mercocresal) one minute before injection resulted in substantially less bacterial counts (14% and 24%) in both needle and cartridge. These clinical findings demonstrate the inapparent means by which pathogenic organisms may be transmitted from patient to patient, as well as the likely introduction of microbes from a patient's own mouth into deep tissue.

To ascertain the technic in common practice today, 1272 practicing dentists were surveyed. Of the 54.2% responding, 11.9% did not attempt any sterilization of injection sites and only 42.5% indicated they routinely used surface antiseptics. Further, it was shown that 88% of the dentists used the same anesthetic cartridge for multiple injections in the same patient and 31.4% said they occasionally used the same cartridge on different patients.

Results of this study, along with surveys representing a cross section of dental graduates from all geographical areas of the country, have focused attention on the critical need for a careful evaluation and reexamination of prevailing technics of local dental anesthetic administration.

PHYSIOLOGICAL RESPONSE TO DENTAL STRESS

The response of the eosinophilic leukocyte in the circulating blood has been used in many studies as a convenient yardstick of the body's reaction to stress. It has been shown for example that a decrease in the number of eosinophils is associated with increased adrenocortical activity that accompanies a state of stress. NIDR investigators reported this year on the use of such procedures in clinical studies of psychosomatic factors in oral disease.

Under the direction of Dr. Irwin I. Ship, Clinical Investigations Branch, 24 adults with extensive dental caries were studied during the course of 235 dental appointments of 45 minute duration. Prior to and 4 hours after each appointment, standard eosinophil counts were done on capillary blood taken by finger puncture.

Catagories of patient classification were made in accordance with the nature of the examination or operative procedure at any given appointment. These included: (1) examination and interview, (2) X-ray examinations, (3) prophylaxis, (4) operative dentistry consisting of amalgam restorations, (5) polishing of restorations, and (6) oral surgery. Prior to all of these procedures, a control base line was established by permitting patients to rest alone in the examination chair for 45 minutes.

Results showed that variation in stress between individuals was greater than in the same individuals, and variation between treatments was greater than during treatments. Adrenocortical activity varied directly with the magnitude of stress involved in the dental procedure; all procedures showing significant differences from control appointments. It was further revealed that the eosinophil levels were not affected by local anesthesia or barbiturate pre-medication in any of the categories of treatment except oral surgery where administration of barbiturates was associated with a relative increase in the eosinophilic level.

CLINICAL ASSOCIATESHIPS IN PERIODONTOLOGY AND ORAL PATHOLOGY

As epidemiology studies continue to demonstrate the high prevalence of diseases affecting the soft tissues of the mouth, increased research emphasis is being focused on the study of periodontal and related oral diseases. It is in this field of dental inquiry that a particularly acute shortage of trained research workers now exists.

Recognizing the outstanding opportunities for advanced research training offered by the clinical facilities at the National Institutes of Health, the uniquely qualified staff of dental and medical specialists, and the great range of patient material available at the Clinical Center, the Dental Institute has extended its scope of activities to include clinical associateship training in periodontology as well as oral pathology.

The development of proficiency and experience in the conduct of clinical research constitutes a principal goal of the associateship programs. Although the basic biological sciences have recognized the important contributions that can be made through a better understanding of collagen tissue and enzymatic and microbiologic factors involved in tissue breakdown, the essential clinical component to advancement of knowledge has been too long neglected. Clinical associates will be encouraged and given the opportunity to carry out research projects which will be closely correlated with related laboratory investigations in such fields as biochemistry and bacteriology. Additional emphasis will be given to participation in regularly scheduled conferences and weekly ward rounds.

GRANTEES SEEK PROTECTIVE COATING FOR TEETH

One approach to the prevention of dental caries is to form a protective coating on the tooth surface. Considerable interest has been shown

in the topical application of the long chain aliphatic amines and silicone compounds to produce a durable and invisible microfilm and thereby reduce the adhesion of dietary foodstuffs serving as substrate for oral microorganisms. NIDR grantees at the University of Alabama reported this year in the Journal of Dental Research on preliminary studies to develop a satisfactory coating for teeth.

Enamel coatings, if successful, must adhere to the tooth surface for a reasonable length of time and be highly resistant to wear. Coatings prepared from organic derivatives of silicone were chosen initially by Drs. R. C. Caldwell, A. Gallagher and R. W. Liggett for evaluation in meeting these basic requirements. Extracted lower incisor teeth from cattle were used throughout the experiment. The teeth were divided into several groups and washed in water and cleaned by lightly brushing with pumice. A number of chemical agents, initially applied to the tooth surfaces, were tested for their ability to enhance the bonding properties of various protective coatings. These so-called "primers" included tetrabutyl orthotitanate and several silicone adhesives. The protective coatings used were prepared from organic derivatives of silicone, phosphone and titanium.

In order to evaluate the primers and protective coatings, all treated teeth were stored in water at room temperature for two months after which they were examined for discoloration, peeling, tacking, and water repellency. Final testing related to a comparison of the adhesiveness of foodstuffs to tooth surfaces with and without protective coating.

Results of the tests showed that application of a primer, especially tetrabutyl orthotitanate, followed by a suitable coating agent, preferably dimethylpolysiloxane is highly satisfactory and holds promise as a potential aid to the control of dental caries.

EPIDEMIOLOGY

EPIDEMIOLOGIST JOINS SURVEY TEAM IN ETHIOPIA

Research has shown that diseases of the oral cavity are most likely not a result of a single agent but rather a plurality of integrated

conditions. Because of this aspect of both a multiple and non-specific etiological concept, it is necessary for dental researchers to isolate and group each of the various causal elements so as to evaluate their interaction in the etiological pattern.

The Interdepartmental Committee on Nutrition for National Defense conducted a health survey in Ethiopia from September 28 to December 5, 1958. Dr. N. W. Littleton, Epidemiology and Biometry Branch, NIDR, served as a member of the special team composed of 13 American scientists and 23 Ethiopian health officers.

During the period of study, approximately 50 locations were visited where food samples, dietary histories, and blood and urine samples were collected for analysis. In addition, a total of 1,400 dental and 5,000 medical examinations were made.

Data collected from dental examinations compliments those recently obtained from similar epidemiological studies in Alaska where ethnic, geographic and climatic conditions, as well as dietary habits, were in marked contrast with those found in Ethiopia. Significant among the findings in Alaska was the comparatively low incidence of caries and periodontal disease in individuals living in primitive regions compared with those from civilized areas. Preliminary data collected in Ethiopia showed the rate of dental caries to be extremely low, and periodontal disease to be very prevalent and severe among young adults. An important contributing factor to the latter finding was the occurrence of unusually heavy deposits of oral calculus.

Information gathered by the survey team will assist the Ethiopian government in developing future health programs in that country.

PROGRAM DEVELOPMENTS

PERIODONTAL DISEASE

With notable progress now realized in the control of dental decay through fluoridation, and a corresponding national increase in average life expectancy through health research, the principal dental advisory groups to the Surgeon General continue to endorse greater financial support for research in periodontal disease.

Today periodontal disease is of major national importance and shows measured potential for early surpassing dental caries as the leading cause of tooth loss in the United States. Although the disease is readily demonstrated in younger age groups, it is primarily associated with the middle-aged and older persons. The significance of this is emphasized when the steadily increasing average age of the nation's population is considered. Periodontal disease is not a self-limiting disorder, since once started it becomes progressively more destructive. Furthermore, the probable loss of teeth is only one manifestation of the disease, as bacteria from periodontal lesions and abscesses may invade the bloodstream and undermine the general health.

A recent study by the Research Committee of the American Academy of Periodontology reported that slightly less than one million dollars is now being spent nationally for periodontal disease research. These figures included over \$136,000 in Armed Forces funds, \$95,000 invested by private firms; and the balance expended by the Public Health Service, which in the past fiscal year, channeled approximately one-quarter of all NIDR research funds into laboratory and clinical studies of periodontal and related oral diseases. Approximately \$700,000 in research grants were awarded by the Dental Institute for 53 separate projects in seventeen colleges and universities in ten states where a multi-disciplinary attack is being carried out in the fields of bacteriology, biochemistry, histopathology, and epidemiology. In fiscal year 1960 further expansion to 65 projects amounting to \$835,000 was seen for research on dental calculus; the role of diet and nutrition; bacteriological studies; and, the physiology, biochemistry, and morphology of periodontial tissues.

CONFERENCE ON
CONGENITAL
ANOMALIES

Fundamental research on cleft-palate and other congenital anomalies involving growth and development of the facial region

has long been a neglected area in dental as well as in medical research. Recognizing the essential role that dental research plays in this important field of health research, the National Institute of Dental Research sponsored a conference of dental and medical specialists, assembled for the first time, to discuss congenital anomalies of the face and associated structures.

Although structural growth deformities of the face are primarily an oral problem, there is a large diversity of professional interests involving oral and maxillofacial surgery, orthodontia, speech pathology and therapy, otolaryngology, psychology and genetics. While the interest in cleft-palate rehabilitation is high and clinical facilities throughout the country for diagnosis and treatment are on the increase, much of the work being done is based on empirical experience rather than on fundamental scientific information.

Many factors are known to be implicated in the production of oral congenital anomalies. Evidence is now available to demonstrate that these developmental disturbances should not be regarded as simply a matter of heredity. While inheritance theories on cleft-palate and other anomalies have long been exploited, equally valid theories on environmental influences are being developed from experimental evidence.

These and other questions were discussed in the conference on congenital anomalies held this year at Gatlinburg, Tennessee, under the sponsorship of the Dental Study Section of the NIH. Participating in the grant supported meeting were outstanding authorities in the fields of embryology, teratology, physiology, genetics, medicine, dentistry, speech, social sciences and public health, as well as national foundations.

The broadening support of such research conferences should measurably accelerate progress, through cooperative research, in many of the still unexplored health fields.

EMINENT DENTAL
SCIENTIST HONORED
IN CONGRESS

A testimonial statement honoring Dr. H. Trendley Dean, first Director of the NIDR, was read into the Congressional Record of

September 8, 1959.

Dr. Dean's achievements in the study of fluoridated water and its effect on dental health stand today as perhaps the most significant contribution in the history of preventive dentistry. During his more than 30 years as an officer in the Public Health Service, Dr. Dean directed a wide range of dental research activities both in this country and abroad, and was the first proponent of the epidemiological approach to research on dental diseases. It was through the application of this research technique that Dr. Dean and his co-workers at the National Institutes of Health demonstrated practical public health methods for preventing one dental disorder (mottled enamel) and effectively controlling another (dental caries).

In his long and distinguished career, Dr. Dean has been the recipient of many national and international honors and awards. Included among these are the Gorgas Medal, an award of the Association of Military Surgeons, the Lasker Award and the John M. Goodell Prize of the American Public Health Association. In addition, Dr. Dean is the only dentist ever to have been accorded the honor of delivering the Holme Lecture at the London University Hospital Medical College.

In July of this year, the American Dental Association announced the retirement of Dr. Dean from the office of Secretary of the Association's Council on Dental Research, a position he held since his retirement from the Public Health Service where he served as Director of NIDR from 1948 to 1953.

CHILDREN'S TEETH RECOGNIZED AS STRONTIUM INDEX

The advent of nuclear weapons, the subsequent testing of such devices, and the mounting problem of radioactive waste disposal

have all stimulated great interest in strontium metabolism and the potential deleterious effects of radiation upon human health. A preliminary investigative program to assess Strontium⁹⁰ levels through the study of children's teeth has now been approved by the National Advisory Dental Research Council.

Studies of the deposition of radioactive fall-out indicate that young children acquire more Strontium⁹⁰ than do adolescents or adults. Further, it is considered that acquisition occurs in dental structures during calcification of both deciduous and permanent teeth prior to eruption. In view of this affinity, dental scientists have recognized the systematic study of deciduous teeth as a means of gaining important information about

the level of radiation received at a specific time in the life of these children. In addition, bone specimens for Strontium analysis are difficult to obtain whereas teeth are readily available at all stages of human development.

Public interest in the possible danger from Strontium⁹⁰ fall-out was stimulated this year in the State of Missouri by reports that milk from the St. Louis milk shed contained increased amounts of radioactive strontium. Recognizing the unique opportunity for investigation in this area, the National Advisory Dental Research Council recommended approval of a feasibility study aimed at assessing the level of Strontium⁹⁰ in large numbers of exfoliated, primary teeth obtained from the greater St. Louis area. Under the direction of Dr. John T. Bird, Washington University School of Dentistry, specimen teeth will be classified according to type, geographical location and life span. The life span and calcification period will be recorded for future correlation between Strontium⁹⁰ content and calendar dates of known radioactive fall-out in particular communities. In addition to the collection of base line data on the uptake of Strontium⁹⁰ by dental structures, efforts will be made to correlate the Strontium⁹⁰ content of teeth with that of bone. If such a correlation does exist, a mechanism for estimating the level of radioactive strontium in skeletal tissue will have been demonstrated.

HIGHLIGHTS OF PROGRESS

IN

MENTAL HEALTH RESEARCH

1959

Items of Interest on Program Developments and Research Studies Conducted and Supported by the National Institute of Mental Health

During the year 1959, many of the new mental health programs that were initiated during the past few years began to bear fruit. The expansion of existing areas of research and the development of new types of research have brought an integrated biological, psychological, and sociological approach to the problems of mental illness. There has been increased activity in the biological and sociological disciplines concerned with mental health, along with continued high interest in the pursuit of psychological studies. New efforts have been made in the attempt to interdigitate anatomy, physiology, and behavior, and much progress has been achieved in furthering knowledge about the impact of social institutions on individual psychology.

MENTAL ILLNESS -- THE SEARCH FOR PATHOLOGY

One of the most baffling aspects of research in the field of mental health has been the virtually complete way in which any morphologic basis for mental illness has eluded discovery.

THE PATHOLOGY OF SCHIZOPHRENIA

Following a historical survey of research into the histopathology of schizophrenia,

Dr. Darab K. Dastur, Rockefeller Fellow and Visiting Scientist in NIMH's Laboratory of Clinical Science from 1956-1958, concludes that no specific change in any tissue or system of the body has been demonstrated that can account for the clinical syndrome of schizophrenia. The vast body of literature on the subject consists of pronouncements about anatomic alterations believed to be responsible for schizophrenia and negative reports demolishing this claim.

The literature of four historical periods was scrutinized by the author, who is now at the Tata Memorial Hospital, Parel, Bombay, India. The first period (1913-23) was characterized as a productive decade from the point of view of histopathology, although not a very discerning decade. The nervous, the cardiovascular and the endocrine systems were examined in the search for organic pathogenesis of schizophrenia. The second period (1924-33) was characterized by much theoretical work without any fresh avenues of approach being opened up. The third period (1934-45) was one in which history repeated itself at a more sophisticated level, with the emphasis again being on changes in nervous and vascular tissues. A new concept that arose during this period was one involving the connection between disorders in the gastrointestinal system and pathogenesis of schizophrenia. The last period reviewed (1946-57) revealed a considerable amount of literature on all body systems previously studied--nervous, cardiovascular, endocrine, and gastrointestinal. The only fairly consistent thread of evidence presenting the histological correlate of a clinical picture appears to be in catatonic schizophrenia, particularly of the acute variety. However, pathological descriptions vary considerably from investigator to investigator, and the histologic changes might be merely secondary to the phenomenon of catatonia. Nonspecific histological changes have rarely been found in schizophrenics and, even when detected, appear to be unrelated to the psychological derangement. Dr. Dastur's survey appeared in the AMA Archives of Neurology and Psychiatry.

BIOCHEMISTRY OF MENTAL ILLNESS

More recently, research has suggested that some forms of mental illness may be related to biochemical processes in the brain and nervous system. If these indications can be followed up, significant advances may be made in understanding and treating these important disorders. To establish that changes in brain functions and the occurrence of mental illness do have a definite causal relationship is, however, a complicated and expensive task, demanding an energetic pursuit of promising research leads. As basic research is making increasingly clear, the brain is a highly complex system made up of a great many separate subsystems, each with its own neurophysiological and biochemical characteristics. The intricate and delicate processes constantly going on in each of these subsystems in the brain, as well as throughout the whole central and autonomic nervous system are gradually being discerned. But the vast unanswered question still remains: What are the ties between all these complex chemical processes and the equally complex congeries of the mental illnesses?

One approach to this problem lies in the search for possible psychotoxic substances that may occur in the blood or urine of patients suffering from mental illness. If there is some biochemical derangement in these patients, a careful analysis of their bodily fluids and a comparison of these fluids with those of normal people may reveal the source of the mental illness.

It is extremely difficult, however, to determine whether a certain chemical substance is related to the cause or the product of a mental disease. Also, many extraneous factors, such as diet and incidence of infectious diseases, may account for differences in biochemical processes and reactions between normal persons and mental patients. The past year has seen serious doubt cast on several attempts to relate abnormal body chemistry to mental illness, but new evidences of this relationship continue to appear.

ABNORMAL PROTEIN IN SCHIZOPHRENIC PLASMA QUESTIONED

Malcolm Siegel, G. Donald Niswander, and others at New Hampshire State Hospital have reported that in seven meticu-

lous attempts in their own laboratory (and similar attempts by five other laboratories) it was not possible to confirm that an abnormal protein, "taraxein," reported found in the blood of schizophrenic patients produces symptoms of schizophrenia in normal monkeys and humans. Siegel and Niswander's work, which is being supported by an NIMH grant, raises serious doubts about the work reported in 1957 by R. G. Heath, S. Martens, and coworkers at Tulane University. They reported that "taraxein," a protein isolated from the blood of schizophrenics, produces catatonia in normal monkeys and humans and causes an abnormal EEG pattern in monkeys with septally implanted electrodes. Because of the importance of this finding and its implications for the etiology and treatment of schizophrenia, Siegel and Niswander, using the taraxein-isolation procedure described by Heath, made seven separate attempts to isolate an active material but were never able to confirm Heath's findings. They point out that all conditions of the Tulane work were approximated as closely as possible; even water used to process the blood plasma was adjusted to approximate the salt content and alkalinity of the water used at Tulane. When they failed to reproduce the findings of Heath et al., the investigators at New Hampshire State Hospital studied the effects of saline, copper salts, and ammonium salts to determine whether an artifact may have been involved in the Tulane observations, but again no behavioral effect was observed in monkeys.

Siegel, Niswander, et al. suggest that the findings of the Tulane group may be attributable to some factor or subtle technique of which the experimenters themselves are unaware. Their work suggests a number of necessary controls in the evaluation of "taraxein" as a primary factor in schizophrenia. They report their studies in The American Journal of Psychiatry.

URINARY EXCRETION OF PHENOLIC ACIDS

Investigating reported abnormalities in urinary excretion of phenolic acids by male schizophrenic patients, NIMH scientists Jay D. Mann and Elwood H. LaBrosse from the Laboratory of Clinical Science established that the differences between patients and normal subjects were due to different coffee-drinking habits.

Previous reports that schizophrenics exhibit a general "aromaturia" had aroused interest because the psychotomimetic effects of numerous aromatic organic compounds suggest that schizophrenia may be caused by derangement of the metabolism of a naturally occurring aromatic compound. A statistically significant difference in excretion of four phenolic acids was found between the group of male schizophrenic patients and normal male subjects while both groups presumably were on a uniform diet, the urinary excretion of the schizophrenic group being significantly higher. However, since these same compounds had been reported as metabolic products of substances present in coffee, a careful examination of the coffee-drinking habits of individuals under study was made. It was discovered that the schizophrenics drank significantly larger amounts of coffee than the normal subjects and the lack of similarity in the coffee-drinking habits completely accounted for the differences between these two groups rather than the presence of schizophrenia. This finding emphasizes the importance of a careful search for uncontrolled variables in the presence of metabolic differences, as well as the need to recognize the high sensitivity of paper chromatographic methods to certain dietary variables. The study was reported in the AMA Archives of General Psychiatry.

ABNORMAL BLOOD FACTOR IN PSYCHOTICS

J. R. Bergen, R. B. Pennell,
H. Hoagland (an NIMH grantee),
and H. Freeman of the Worcester

Foundation for Experimental Biology recently presented evidence showing that blood plasma fractions from psychotic patients markedly affected behavior in rats. Injections of either normal or psychotic fractions prolonged rats' performance on a rope-climbing test, but the rats given psychotic fractions showed delays twice as great as those seen in rats given normal fractions. These findings contribute further evidence for the existence of an abnormal factor in the blood of psychotics.

Blood fractions obtained from normals and from actively hallucinating, recently admitted psychiatric patients were injected into rats. Both fractions increased the time required for the rats to climb a rope, but the psychotic fractions were approximately twice as effective in causing delay as the normal fractions. After the two types of blood plasma were placed on opposite sides of a cellophane membrane to allow the selective dialysis of small molecular materials, the dialyzed normal blood fraction produced a climbing time delay at least equal to that previously produced by the undialyzed psychotic fraction. This dialyzability of the "active" portion indicates that it is probably not a protein but a small molecule capable of being attached to a protein. The studies were reported at the meeting of the Federation of American Societies for Experimental Biology.

BIOCHEMICAL THEORIES OF SCHIZOPHRENIA

In a critical review of biochemical trends in schizophrenia research during the past few

years, Dr. Seymour S. Kety, Chief of NIMH's Laboratory of Clinical Science, concludes that although the evidence for genetic factors in the etiology of schizophrenia is compelling, the signposts pointing the way to their discovery are at present quite blurred. He notes that the genetic and environmental approaches to the etiology of schizophrenic psychoses are not mutually exclusive. One hypothesis compatible with all the genetic as well as psychosocial disciplines is that schizophrenic disorders are the result of the operation of environmental factors on a genetically determined predisposition. Dr. Kety also calls attention to new knowledge about the nervous system, thought processes, and behavior as an extremely valuable potential by-product of the current interest in biochemical studies of schizophrenia.

Dr. Kety begins his review, published in two issues of Science, of current biochemical theories of schizophrenia with a discussion of the sources of error in this type of research. These include the large number of variables involved, the lack of evidence that all forms of schizophrenia have a common etiology, the errors involved in sampling heterogeneous populations, and the problems involved in the presence of infections, nutritional deficiencies, and even different patterns of intestinal flora among groups of hospitalized patients. He also calls attention to the fact that some physiological and biochemical changes are secondary to the psychological and behavioral state of the patient, and he notes that measurements of changes in the mental state or behavior are highly subjective and that symptoms are extremely variable and responsive to nonspecific factors in the milieu.

The review contains a brief description of the program of biochemical research being carried out in the Institute's Laboratory of Clinical Science. This program has been specifically designed to minimize the sources of error as much as possible while increasing the opportunity to detect, and to correlate with psychiatric and behavioral information, any true biological characteristics that may exist. To date, the schizophrenic patients studied, either individually or as a group, have shown little abnormality in the biological studies completed.

Dr. Kety examines the various studies attempting to show relationships between schizophrenia and disorders in oxygen metabolism, amino acid metabolism, or metabolism of epinephrine; the theories related to ceruloplasmin, taraxein, and serotonin deficiency; and the attempts to correlate genetic factors and schizophrenia.

In his summary, Dr. Kety points out that genetic factors may operate at various possible levels: through some ubiquitous enzyme system to effect general changes in one or another metabolic pathway detectable by studies on blood or urine, by highly localized enzymatic changes within the brain, or by inappropriate interconnections or interactions between chemically normal components. He emphasizes the need for new hypotheses, mentioning gamma-amino-butyric acid and amines other than serotonin and norepinephrine as substances about which to construct working hypotheses, and points to the need for developing new techniques which will yield information about processes occurring within the psychotic brain. He cautions against premature optimism that a simple and fundamental biochemical defect in schizophrenia has been discovered or will be in the near future, but finds encouragement in the rapid growth of new biological knowledge related to behavior and mental state.

PSYCHOPHARMACOLOGICAL RESEARCH

Another strong impetus to the discovery of biochemical mechanisms in mental illness is found in the intensive research now under way on the new drugs used to treat mental illness.

NIMH GRANTEE RESEARCH

The effectiveness of the Institute's Psychopharmacology Service Center's effort to stimulate research in this area is perhaps reflected in the fact that at the September 1959 meeting of the American Psychological Association approximately one-third of the papers directly relevant to psychopharmacology reported work which is being supported by NIMH grants. These seven papers covered a wide range of experimental research, much of which has clinical implications. Some described new or modified techniques which might be used to screen drugs for behavioral or psychological effects. Others reported investigations

of the effects of specific drugs on the behavior or psychological test performance of human or animal subjects. Some dealt with studies of the sites or mechanisms of action of specific drugs, some with methodological problems. One described experimental work on a test of hypothalamic excitability that may prove helpful in psychiatric diagnosis.

Alberto DiMascio and Jonathan Brown, Massachusetts Mental Health Center, described a study of the effects of drugs on anxiety in normal human subjects. It was found that pheryltoloxamine and meprobamate decreased anxiety as measured by test performance, but that reserpine and secobarbital had no significant effect on anxiety.

Solomon D. Kaplan, University of Kansas Medical Center, described a visual test of hypothalamic excitability. His experimental work indicates that the test might be used as a diagnostic tool in psychiatry.

David Krech, Mark R. Rosenzweig, Edward L. Bennett, and Charles L. Longueil, University of California, reported studies showing that genetic differences in animal strains are an important aspect of animal-behavior studies. Using descendants of two specially bred strains, these workers found significant differences between the two groups of rats in learning on various tests, in brain cholinesterase activity, and in brain weight.

Donald M. Krus and Seymour Wapner, Clark University, reported a study in which they found that lysergic acid diethylamide causes an adult's visual perception to become more like that of a child. They interpreted these findings as supporting what they termed the "regression hypothesis", that LSD works as a primitivizing agent, causing a regression to more childlike perception of height of the horizon.

The paper by Ausma Rabe and R. W. Gerard, University of Michigan, described a study of drug effects on the memory fixation time of rats. They found that increasing doses of meprobamate protected against the disrupting effects of electroconvulsive shock. Barbiturates, in contrast, increased the ECS effect but did not slow learning.

R. P. Travis and J. Olds, University of Michigan, reported an investigation of drug effects in rats given electric stimulation through electrodes implanted in various parts of the brain. They found that the effect of chlorpromazine or morphine on the rat's behavioral response to shock differed when shock was applied to different areas of the brain.

Herbert Barry, III, and Neal E. Miller, Yale University, reported a new technique for measuring the degree of fear or pain which causes a rat to take action to prevent or eliminate the pain. The method provides a reliable, sensitive measure of both approach (hunger drive) and avoidance (shock intensity) in rats. It is also sensitive to effects produced by different types of drugs, and may therefore be valuable as a technique for determining certain behavioral effects of new psychoactive compounds. Its promise as a new drug-screening technique is another good example of the potential application of basic research findings to the solution of practical problems. Barry has also devised a "telescope alley" in which the distance of the food cups from the starting box and the height of the food cups are associated with intensity of electric shock. After the rats show consistent performance patterns without drug, they are tested following administration of drug. In a recent study, chlorpromazine showed no effect on either approach or avoidance. Alcohol decreased avoidance, whereas both caffeine and methamphetamine increased avoidance. That is, with alcohol the rats withstood a greater number of shocks in order to obtain food; with either caffeine or methamphetamine (particularly the latter), the rats withstood far fewer shocks--as compared with nondrug conditions--before they stopped running for food. Some of these results were also reported at the meetings of the Federation of American Societies for Experimental Biology.

BIBLIOGRAPHY OF PSYCHOPHARMACOLOGY

Psychopharmaca, published recently by the Public Health Service, covers the literature on psychopharmacology

from 1952-1957. Approximately 2500 articles are included which are "concerned with the effect of psychopharmacologic agents on the psychologic, behavioral, and encephalographic reactions of normal subjects, patients, and laboratory animals." Prepared by Anne E. Caldwell, M.D., National Library of Medicine, at the request of the Psychopharmacology Service Center, NIMH, it provides an invaluable tool to scientists and others in this relatively new field. The bibliography contains a drug index, a subject list of drugs by generic drug name, an ancillary subject list of special conditions, and an author list. All the material is carefully cross-referenced.

ANIMAL-SCREENING TECHNIQUES

The Institute's Psychopharmacology Service Center has held three informal meetings with represen-

tatives from industry and academic research groups to review and evaluate current methods of accumulating animal laboratory data on psychopharmacological agents. The meetings confirmed the need for continued effort to develop much better, more sensitive and more accurate techniques for (1) early identification of those

drugs with some indication of psychoactivity, and (2) predicting from animal studies what effects these drugs will have on humans in general and particular types of mental patients specifically.

The topics of the three meetings were, respectively, operant conditioning techniques in drug screening, non-operant approaches to animal screening of psychoactive drugs, and neurophysiological techniques in drug screening. The consensus of the meetings was that some of the currently used screening techniques are promising and valuable, but that none of them is entirely satisfactory or reliable. The participants felt that at the present time one must rely too greatly on the "happy accident" and on unquantifiable observations of animal behavior.

CLYDE MOOD SCALE

The Clyde Mood Scale, a tool for measuring the subjective effects of psychoactive drugs developed

by Dr. Dean J. Clyde of the Institute's Psychopharmacology Service Center, is proving effective in evaluating the results of these drugs. This test consists of a number of adjectives on cards which can be sorted by subjects to describe how they feel. When used with two groups of office workers, the Mood Scale distinguished between those with mild neurotic complaints and those without such complaints. A meprobamate pill affected workers who often turned up for sick call with neurotic complaints differently than did a placebo. Office workers who did not turn up for sick call did not react to a meprobamate pill differently from the way they did to a placebo. The Clyde Mood Scale showed that the meprobamate pill affected the way the neurotic workers felt but did not have any effect on the "normal" workers.

BASIC RESEARCH WITH PSYCHOACTIVE DRUGS

Along with the work to evaluate the clinical effects of these drugs is research on their basic

mechanisms of action. Interesting leads have come from research on reserpine and iproniazid, to cite just two examples. It has been found that serotonin and norepinephrine are naturally occurring substances in the brain. Reserpine flushes both serotonin and norepinephrine out of the brain, and also makes a person tranquil and quiet. Iproniazid, on the other hand, causes serotonin and norepinephrine to accumulate in the brain, and on the behavioral side sometimes relieves depression and sometimes produces psychotic excitements. But it is still unclear whether or not the effects of these two drugs on the amount of serotonin and norepinephrine in the brain is directly and unequivocally related to the changes in behavior produced by the drugs. When we find out what these and other psychiatric

drugs really do to the chemistry of the body, we will also have learned much more about the biochemistry of mental illness. To assist in the development of such research the Institute's Psychopharmacology Research Center awarded a \$25,000 contract to the Regis Chemical Company of Chicago on September 1, 1959, to support work on the synthesis of certain indole derivatives of interest to investigators engaged in basic psychopharmacological research. The compounds to be synthesized have a close, structural relation to LSD and bufotenine and will be tested for psychopharmacological effects.

Psychotomimetic substances--chemicals that can produce transient psychotic-like states in normal people--are being used as tools to uncover biochemical factors affecting behavior. These substances, which include such diverse chemical compounds as lysergic acid diethylamide, mescaline, sernyl, benactyzine, and a series of 3-N-substituted piperidyl benzilates, make normal people have all sorts of psychotic symptoms such as hallucinations, delusions, and feelings of unreality and dissociation. If we can discover why chemical compounds cause these changes in behavior, we will have some significant clues to the chemical structure and processes of natural substances in the body that, under certain circumstances, may give rise to mental illness.

PSILOCYBIN AND LSD-25

A comparative study of the reactions induced by psilocybin and lysergic acid diethylamide

(LSD-25), conducted by Dr. Harris Isbell, Director of NIMH's Addiction Research Center at Lexington, Kentucky, revealed that both hallucinogenic drugs have similar effects. Psilocybin, a synthetic compound structured after the active ingredient in an intoxicating mushroom used by Indians in Mexico, is chemically related to serotonin and to bufotenine. Because of this, and because it is a much simpler compound than LSD, it may prove to be an important tool in biochemical studies on the role of serotonin in brain function.

Psilocybin (O-Phosphoryl-4-hydroxy-N-dimethyltryptamine) is chemically related to serotonin (5-hydroxytryptamine) which is believed to play an important role in the function of the central nervous system. This similarity has led to the speculation that psilocybin may cause an abnormal state by interfering with the actions, synthesis, disposition or metabolic degradation of serotonin. Other researchers have postulated that LSD-25 and other psychotomimetic agents might act through such mechanisms.

In Dr. Isbell's study, reported in the first issue of the new journal Psychopharmacologia, both LSD and psilocybin caused elevations in body temperature, pulse and respiratory rates, and systolic blood pressure. Threshold for elicitation of the kneejerk was decreased by both drugs. After both drugs, abnormal mental states characterized by feelings of strangeness, difficulty in thinking, anxiety, altered sensory perception (particularly visual), elementary and true visual hallucinations, and alterations of body image were reported by the subjects. The effects of psilocybin did not persist as long as those of LSD. LSD is 100 to 150 times as potent as psilocybin. The similarity of reactions induced by LSD and psilocybin suggest that a common biochemical or physiological reaction is responsible for the effects of these two drugs.

Psilocybin was isolated by Hoffman and associates from the mushroom, Psilocybe mexicana Heim, which has been used by the Mexican Indians since pre-Columbian days in their religious rites. The chemical constitution and synthesis of psilocybin was announced in 1958 by Hoffman and associates. It is manufactured by Sandoz and Company. It is a psychotomimetic drug of basic research interest for those who wish to induce psychotic-like states. It is not available commercially.

PSYCHOTOMIMETIC AND ANTI-SEROTONIN POTENCIES OF LSD

made by Drs. Harris Isbell, E. J. Miner and C. R. Logan, at the NIMH Addiction Research Center at Lexington, Kentucky. Examination of the effects of 13 congeners of LSD-25 demonstrated that high potency as a serotonin antagonist in isolated smooth muscle preparations was not correlated with high potency as a psychotomimetic.

Further insight into the psychotomimetic properties of lysergic acid diethylamide (LSD-25) was contributed by a recent study

Recent postulations have indicated that LSD induces psychosis by creating a relative deficiency of serotonin within the brain or conversely by acting like serotonin in the brain. Serious doubt about the deficiency hypothesis was created by the finding that D-2-bromodiethylamide of lysergic acid (BOL-148), which is as potent as or more potent than LSD in blocking serotonin in isolated smooth muscle preparations, was not a psychotomimetic drug or only a very weak one. Availability of a number of congeners of LSD-25 with varying potencies as antagonists of serotonin on isolated uterine muscle of the rat made possible a more detailed examination of the relationship between potency of such drugs as serotonin antagonists and their potency as psychotomimetics. The data in this study, reported in the first issue of

Psychopharmacologia, do not support (but also do not disprove) the serotonin deficiency hypothesis of the LSD psychosis.

METABOLIC STUDIES

Recent years have seen the dramatic and significant discovery of a number of neurochemical substances that appear to play major roles in the activity of various brain cells and the transmission of nerve impulses. Acetylcholine, serotonin, epinephrine, norepinephrine, histamine, and gamma-aminobutyric acid are a few examples. The enzyme systems responsible for synthesizing and destroying these substances are now understood, at least in part. The principal pathways through which the substances are metabolized are under detailed investigation in many laboratories, and the distribution of the substances and their related enzymes in various parts of the brain and nervous system are being determined.

Furthermore, the metabolic pathways through which glucose passes to provide energy to the brain are becoming better known. Recent research has proved for the first time that not only glucose, but a variety of amino acids and other chemicals are also metabolized in the brain. The characteristics of the blood-brain barrier, which prevents many substances injected into the blood stream from entering the brain and strictly limits the role of their entry, is also being better understood. The effects of many different substances, such as hormones, which are manufactured in the body and which almost certainly have a relation to the biochemistry of the nervous system, are being explored.

METABOLISM OF EPINEPHRINE

The principal metabolic pathway of the important hormone, epinephrine, has been determined in the human system. NIMH investigators in the Laboratory of Clinical Science, studying animal and human subjects, have found that catechol-O-methyl-transferase, rather than monoamine oxidase, is the enzyme mainly involved in the termination of epinephrine's action. Since epinephrine is intimately related to nervous system functioning, the finding bears significantly on the possible role of biochemical disturbances as a cause of psychosis.

Although for many years monoamine oxidase had been considered to be the primary enzyme in the metabolism of epinephrine, Dr. Julius Axelrod of the NIMH Laboratory of Clinical Science has found evidence that the principal metabolic pathway of epinephrine in man is O-methylation to metanephrine. The extent of the conversion to metanephrine, a physiologically inactive compound, indicated to Dr. Axelrod that the enzyme responsible for this

reaction, catechol-O-methyl-transferase, is the enzyme mainly involved in the termination of action of epinephrine. He confirmed these findings in man in collaboration with Drs. Elwood M. LaBrosse and Seymour S. Kety of the same Laboratory. It was further indicated that the role of monoamine oxidase in epinephrine metabolism is mainly in the deamination of metanephrine. This would explain the observations that iproniazid, an inhibitor of monoamine oxidase, does not prolong the physiological actions of epinephrine in the living body.

It follows that the principal pathway of epinephrine's metabolism in man is O-methylation to metanephrine, which in turn is conjugated and deaminated. This, together with the lack of physiological and psychological action of 3-O-methyl catechol amines previously observed with normetanephrine and metanephrine, points to catechol-O-methyl transferase as the principal enzyme for the inactivation of epinephrine. Recognition of epinephrine's important role in mammalian physiology dates back at least half a century, but the metabolic fate of this compound has been poorly understood heretofore. The new studies, reported in Science, provide a clearer picture of metabolic processes affecting nervous system functioning and bodily responses to stress, and may bear on the possibility of a biochemical basis for schizophrenia or other psychoses.

NEW METABOLITE OF EPINEPHRINE

Dr. Julius Axelrod of NIMH's
Laboratory of Clinical Science
has further elaborated his

important contribution to the metabolism of epinephrine. In collaboration with Drs. Irwin J. Kopin and Jay D. Mann (also of the Laboratory of Clinical Science), he has discovered a new metabolite of this important hormone, 3-methoxy-4-hydroxy-phenylglycol, and demonstrated its presence in normal urine and in large amounts in the urine of patients with pheochromocytoma. Substances which inhibit the enzyme, catechol-O-methyl-transferase, and potentiate the action of epinephrine in vivo were demonstrated. The tranquilizing drugs, chlorpromazine and reserpine, were found to speed the destruction of epinephrine in vivo, a finding which may be related to the psychopharmacologic action of these agents. This work was reported in Biochimica et Biophysica Acta and in Nature.

INHIBITOR OF O-METHYLATION OF EPINEPHRINE

Further evidence of the way in
which the body inactivates
epinephrine and norepinephrine
was provided in a study focused

on the contribution of pyrogallol to this process. In a report published in Science, Dr. Julius Axelrod of the NIMH Laboratory

of Clinical Science and Dr. Marie-Jeanne Laroche, NIH postdoctoral fellow now at the Institut Pasteur in Paris, present evidence pointing to catechol-O-methyl-transferase as the enzyme mainly involved in the inactivation of the neurohumor norepinephrine in the sympathetic nervous system.

Pyrogallol inhibits the O-methylation of epinephrine and norepinephrine by catechol-O-methyl-transferase in vitro as well as the metabolism of these catecholamines, and the formation of their O-methylated metabolites, in the intact mouse. Since pyrogallol also prolongs the physiological effects of epinephrine, it is suggested that catechol-O-methyl-transferase terminates the actions of the catecholamine hormones.

PHENYLALANINE TO TYROSINE

Further studies on the mechanism of the enzymatic conversion of phenylalanine to tyrosine, con-

ducted by Dr. Seymour Kaufman of NIMH's Laboratory of Cellular Pharmacology, have brought increased understanding of the metabolic fate of this substance whose faulty metabolism results in phenylketonuria, a condition often leading to a severe form of mental retardation.

One study, reported in the Journal of Biological Chemistry, dealt with the enzyme system which catalyzes the oxidation of phenylalanine to tyrosine. The system involves two enzymes; one purified from rat liver and the other from sheep liver extracts, in addition to oxygen, TPNH, and any one of several tetrahydropteridines.

The other study, reported in Biochimica et Biophysica Acta, dealt with the participation of tetrahydrofolic acid in the enzymic conversion of phenylalanine to tyrosine. Dr. Kaufman has identified dihydrofolic acid as the product of oxidation of tetrahydrofolic acid by the phenylalanine oxidase system. Tetrahydrofolic acid serves as an electron donor in this reaction and appears to be similar in structure and function to the natural, and as yet unidentified cofactor.

ENZYMATIC N-METHYLATION OF HISTAMINE

NIMH scientists, Drs. Donald D. Brown, Julius Axelrod, and Robert Tomchick have isolated

and described the properties of imidazole-N-methyl-transferase, which they believe to be the enzyme primarily responsible for the inactivation of histamine.

The compound histamine is a natural constituent of many tissues, both plant and animal, where it apparently exists in combination with some constituent of the cell. It has been variously proposed that histamine plays a role in a variety of physiological and pharmacological processes, such as the induction of labor and the secretory activity of the gastric glands and pancreas, and that it is the ultimate noxious agent in various types of allergies, surgical shock and peptic ulcer. Histamine can be released from cells by noxious stimuli of either a chemical or physical nature. Total release of all the bound histamine in the body would prove fatal. The mechanism involved in the release of bound histamine is not fully known. Elucidation of this mechanism will be a major contribution to an understanding of the reaction of cells to a variety of noxious agents.

For many years it was thought that deamination was the process whereby histamine was metabolized and that histaminase was the enzyme responsible for this process. Dr. Schayer, while at Northwestern University, found that methylation and not deamination was the process responsible for the metabolism of histamine. He demonstrated this in man and several other species, including the cat, the dog, and the mouse.

NIMH investigators have now discovered the enzyme imidazole-N-methyl-transferase which is responsible for the methylation of histamine. This enzyme is widely distributed in a variety of species and organ tissue. Particularly noteworthy is its high concentration in the central nervous system. Drs. Brown, Axelrod, and Tomchick believe that this enzyme is concerned with termination of the action of histamine at various organs at which histamine exerts its effects. S-adenosylmethionine is required for the N-methylation of histamine. It is a key compound in the metabolism of many important physiologically active amines, such as histamine, adrenaline, and noradrenaline. The findings were reported in the English scientific journal, Nature.

METABOLITES OF HISTIDINE

Drs. Donald D. Brown and
Marian W. Kies of NIMH's
Laboratory of Clinical Science

have discovered several new metabolites of histidine in addition to previously known and isolated compounds.

In one study, reported at the 1959 meetings of the Federation of the American Society of Biological Chemists, the investigators described an unknown compound which appeared among the urinary

metabolites following intravenous injection of uniformly radioactive C-14-L-histidine in a female Macaca mulatto monkey. The new metabolite, which represented at least 14 percent of the urinary radioactivity, had marked acidic properties and was not on the histamine or urocanic acid pathway of histidine degradation. Although the unknown compound still contained the second carbon of the imidazole ring of histidine it did not possess an intact imidazole nucleus.

Several other related studies by these investigators and their colleagues in the Laboratory of Clinical Science on the mammalian metabolism of histidine have been published in the Journal of Biological Chemistry. One paper describes the enzymatic formation of L-hydantoin-5-propionic acid from histidine via the urocanic acid pathway. Another deals with the enzymatic formation, stabilization, purification, and properties of 4(5)-imidazolone-5(4)-propionic acid, the product of urocanase activity. A third report describes the method which they have developed to separate and measure the radioactive metabolites of L-histidine-C¹⁴, in the urine of the monkey, the human, and the rat.

EFFECTS OF PYRIDOXINE WITHDRAWAL

In a study involving collaboration between scientists Drs. Louis Sokoloff, Niels A. Lassen, Guy

M. McKhann, Donald B. Tower, and Wayne Albers, at NIMH and NINDB, a six-year old child, afflicted with a rare type of epileptic seizure that can be prevented by pyridoxine administration, was tested to determine brain metabolism during seizure activity and immediately following clinical "cure" by pyridoxine administration. Since the "cure", representing a prompt discontinuation of the fits and a return of the brain waves (EEG) to normal, takes place within a few minutes of the pyridoxine administration, one can make a crucial comparison of brain metabolism in the two states over a very short time period in the same individual.

The six-year old child who was the subject of this study developed its seizures four hours after birth. The child did not respond to the usual anticonvulsant therapy but the seizures were controlled by high doses of pyridoxine. The child has been maintained free of seizures since two years of age by daily doses of pyridoxine.

Cerebral blood flow (CBF), cerebral oxygen consumption, arterial-cerebral venous oxygen and carbon dioxide differences, and respiratory quotient were determined during a period of seizure activity, and within a few minutes after intravenous administration of pyridoxine, by which time clinical manifestations of the seizure had disappeared and the EEG had returned to a normal pattern. During the seizure, CBF, cerebral oxygen consumption,

arterial-cerebral venous oxygen and CO₂ differences, and respiratory quotient were markedly decreased. Pyridoxine recovery resulted promptly in a slight rise in CBF, a significant increase in cerebral oxygen consumption, and a return of the arterial-cerebral venous oxygen and CO₂ differences and RQ to normal. Since the increase in oxygen consumption amounts to two standard deviations beyond the mean, it may be assumed to be an effect of pyridoxine. These results suggest that pyridoxine deficiency seizures resemble those of insulin hypoglycemia and that, in pyridoxine deficiency, there may be an analogous interference with the supply or utilization of essential substrates for brain metabolism. Convulsions caused by pyridoxine antagonists in animals have been associated with decreased levels of gamma-aminobutyric acid, and such convulsions have been arrested by administration of this agent. Other evidence suggests that gamma-aminobutyric acid may function as an inhibitory neurohumoral transmitter substance, and that it also may play a role in normal metabolic oxidations in the brain. The study was reported in Nature.

METABOLIC STUDIES OF DRUG ACTION

BIOCHEMICAL FACTORS

In a paper read before the German
Pharmacological Society in Basle,
Switzerland on October 2, 1959,

Dr. Julius Axelrod, Chief of the Section on Pharmacology in NIMH's Laboratory of Clinical Science, summarized current knowledge about the biochemical factors involved in the metabolism and pharmacological activity of drugs. Dr. Axelrod himself has contributed a number of highly important findings to this field.

Knowledge concerning the biochemical changes that a pharmacological agent undergoes in vivo and the factors influencing these changes may be of considerable importance in the understanding of drug action, the elucidation of new biochemical phenomena, as well as serving as a guideline in the synthesis of more effective and safe drugs.

Most drugs undergo chemical change in the body to either pharmacologically active or inactive products. A wide variety of biochemical changes, including decarboxylation, acetylation, reduction, oxidation, N- and O-dealkylation and N- and O-methylation, can form active metabolic products. However, depending on the structure of the drug, these reactions may also form inactive metabolites. Other metabolic changes which generally produce inactive metabolic products include deamination, hydrolysis, and conjugation reactions.

Dr. Axelrod, while he was with the NHI, was the first to describe a family of enzymes, localized in the microsomes of the liver, which are responsible for metabolizing a wide variety of drugs. Dr. Rudi Schmid (formerly of NIAMD) and Dr. Axelrod, in their work on glucoronides (an important means by which the body inactivates drugs), discovered that a defect in the enzyme which forms glucuronic acid is the cause of the disease congenital non-hemolytic, non-obstructive jaundice. This is an example of how the study of drug metabolism can help in uncovering the cause of disease.

Although compounds are ultimately transformed by enzymes, there are many factors that modify enzyme activity such as inhibitors, activators, tissue localization, vitamins and hormones. Many drugs owe their long-lasting action to storage in certain tissues; this protects the drug from enzymatic destruction. The action of many drugs can also be prolonged by substances which block the enzymes from acting. Other substances, which increase enzyme activity, can speed up the metabolism of many drugs. There are considerable species differences in the metabolism and action of drugs, owing primarily to species differences in enzyme activity. Many hormones, vitamins, and other foreign compounds affect drug metabolism by enhancing or inhibiting enzyme activity.

Repeated administration may result in the inactivation of certain drugs, notably narcotic drugs. This phenomenon is known as tolerance. Dr. Axelrod found striking similarities between the "analgesic" receptors for narcotic drugs and the enzymes that N-demethylate these compounds. This parallelism suggests a mechanism for the development of tolerance to narcotics.

GREENHOUSE FACILITY

A greenhouse recently completed on the grounds at NIH makes possible further intensive investigations by NIMH scientists of the biosynthesis and the metabolism of plant products. This research is of vital pharmacological importance.

Comparatively little is known about the biosynthetic pathway, the nature of the precursors and breakdown products, and the enzymes and coenzymes involved in the metabolism of compounds such as reserpine, the lysergic acid alkaloids, the opium alkaloids, and many other compounds of direct importance to the field of mental health. The greenhouse makes it possible to obtain specialized compounds by feeding appropriate precursors and metabolic building blocks to growing plants. Furthermore, it is possible to study the role of alkaloids and other nitrogenous compounds which are closely related to the structures of compounds known to act as vitamins, cofactors, and essential cell constituents in micro-organisms and higher vertebrates. Increased understanding of the

biochemical and physiological functions of alkaloids and other compounds in plants will contribute to the field of biological knowledge in general and to the understanding of pharmacological effects in particular.

A FUNDAMENTAL ENZYMATIC MECHANISM

Utilization of plant materials to elucidate the biosynthetic pathways of various compounds has brought

new information about transmethylation, a process which now appears to be important for all forms of life. The study from which this finding resulted is part of a program of research being conducted by NIMH's Laboratory of Cellular Pharmacology, in the greenhouse facility opened on the grounds at NIH in the spring of 1959. Dr. S. Harvey Mudd of the Laboratory reported on his work in Biochimica et Biophysica Acta.

In the biosynthesis of the alkaloids N-methyltyramine, hordenine and gramine by cell-free extracts of barley or millet, the methyl group of these compounds is donated by S-adenosylmethionine. Several other compounds which have been suggested in the literature as possible methyl donors are inactive in this system. These findings were extended in a series of experiments in which it was shown that barley can synthesize S-adenosylmethionine identical to that found in vertebrates even to the extent of having the same stereochemical configuration about the asymmetric sulfur and alpha-amino carbon atoms. Together, these facts very strongly indicate that the predominant pathway of plant transmethylation lies through S-adenosylmethionine just as it does in vertebrates and microorganisms.

These findings illustrate once again the value of studying fundamental enzymatic mechanisms in whatever biological material is most convenient with the assurance that the facts in a given form may well apply to widely divergent forms. Although transmethylation is apparently extremely important for all forms of life, the enzymatic mechanisms at work here are imperfectly understood and it is hoped that further work with botanical systems may clarify details of the process.

A matter which requires further exploration is suggested by the structural resemblance of two of the particular plant alkaloids studied (N-methyltyramine and hordenine) to the adrenal medullary hormones of mammals, and of a third (gramine) to mammalian serotonin. If the role, as yet unknown, of these compounds in plant metabolism can be elucidated, we may gain thereby an important lead to discovering the role of these neurohormones and of chemically related hallucinogenic materials.

The formation of the alkaloids now being studied is known to be under not only genetic control but under other controls as well, so that the formation occurs in a dramatic outburst at a specified stage of ontogenesis and in restricted types of tissues. It seems not unlikely that a study of the interplay of the control mechanisms which are at work here will give insight into the important question of how enzyme formation and activity is governed in higher organisms. The genetic, environmental, tissue specific and hormonal factors cooperating in this system are undoubtedly complex but it is hoped that the relative ease of experimental control of the plant will aid considerably in work on this question.

BRAIN AND BEHAVIOR

Intensive investigations of the physiological and anatomical characteristics of the brain and central nervous system are in continuous progress. The importance of this type of research is being recognized on a world-wide basis. Many studies are bringing important new knowledge about the functioning of specific brain centers and the effects of localized brain damage.

NEW INTERNATIONAL BRAIN RESEARCH ORGANIZATION

Dr. Paul MacLean, Chief, Section on Limbic Integration and Behavior, NIMH, is one of four scientists

from the United States selected for membership in the Provisional Committee on Physiology of a proposed new international Interdisciplinary Brain Research Organization. Fourteen countries are represented on the Committee. Members of the Committee were selected on the basis of their international reputations in their respective specialties. The broad purposes of the proposed new organization are to provide leadership in interdisciplinary brain research throughout the world, to serve as a clearinghouse for information in this field, to plan symposia and international study groups on limited aspects of cerebral physiology, and to provide the framework for creating traveling and residential fellowships to enable specialists of different scientific disciplines and geographic areas to do collaborative research on the brain. The first meeting of the Provisional Committee was held in France on October 4, 1959.

The proposed Interdisciplinary Brain Research Organization (IBRO) was organized through the auspices of the International Federation of EEG Societies and Clinical Neurophysiology. It is registered with UNESCO and its status with UNESCO will be voted upon in 1961.

Because of the prestige of the members of the Provisional Committee on Physiology of IBRO, the organization has the potentiality of becoming a great force in influencing the course of research in the field of neurology and mental health. There will be seven panels, representing other scientific disciplines such as neuro-anatomy, neurochemistry, and neuropharmacology, selected as part of the over-all organization of IBRO.

BRAIN FUNCTION AND INTERRUPTION OF VISUAL PATHWAY

Activity reaching the central nervous system from the visual apparatus markedly affects time patterns in the brain, an NIMH

visiting scientist and two associates in the Laboratory of Clinical Investigations have shown.

Responses of anesthetized cats to electric shocks delivered to the lateral geniculate body--the important relay station in the brain's visual system--were significantly affected by interruption of the optic nerve or electrolytically produced destruction of the lateral geniculate, experimenters Dr. Jean Posternak, Dr. T. Corwin Fleming and Dr. Edward V. Evarts of the NIMH Laboratory of Clinical Investigations have reported. Dr. Posternak is a visiting scientist from L'Institut de Physiologie, Ecole de Medecine, Geneva. Their findings were reported in Science.

Evoked responses were recorded from the surface of the lateral gyrus with a pore electrode. Results indicated that interruption of the optic nerve by freezing or clamping, or electrolytically produced lesions of the lateral geniculate, caused a decrease in variability and an increase in amplitude of the postsynaptic components of the cortical response to geniculate radiation shock. Such lesions also caused a marked decrease in the degree of sub-normality of the surface positive components, though not of the surface negative component, of a test response evoked at various intervals after a previous response. Several experimental variables modified the degree to which recovery was enhanced following interruption of the primary visual pathway. One of these was stimulus intensity, recovery being enhanced more for the responses to supramaximal than to near-threshold stimuli. Also, the recovery cycle in cortical positions outside the major cortical focus of the stimulating electrodes showed less elevation following lesions of the lateral geniculate or optic nerve than did the recovery cycle at the maximal cortical focus.

This experiment, like recent studies of disturbances of psychological functions caused by various kinds of sensory deprivation, offers new illustration of the high degree to which function of the brain is dependent on a continuous flow of information from the outside world.

EFFECTS OF SLEEP AND AROUSAL ON CORTICAL RESPONSE

Evidence indicating that the visual centers of the brain show more electrical activity during sleep than during waking states has been developed in a series of neurophysiological studies conducted by scientists in the NIMH Laboratories of Clinical Science and Neurophysiology.

Studies were conducted by Drs. T. C. Fleming, P. R. Huttenlocher, and E. V. Evarts on cats with small electrodes permanently placed in the visual area of the brain. From these electrodes recordings were made of the electrical activities of the brain during periods when the cats were asleep and awake. Observations have shown that during the waking state there was a marked increase in the duration of the inhibitory forces which play upon the visual cortex following stimulation. This finding suggests that sleep is associated with an alteration of the pattern of cerebral activity, rather than with the absence of activity in the brain. The findings of this study are in agreement with previous theories that the coordinated brain activity required for normal waking behavior is partially dependent upon continuous inflow of controlling (inhibitory) nerve impulses to the sensory mechanisms of the brain. The investigators reported their findings at the meetings of the Federation of American Societies for Experimental Biology.

RECORDINGS FROM SINGLE HIPPOCAMPAL NEURONS

An NIMH comparative study of the electrical activity of single neurons in the hippocampus has added new and important information about the epileptic susceptibility of this region of the brain. In their analysis of the nature of the electrical responsivity of these neurons and their tendency to spontaneous and physiologically evoked discharge, Drs. Eric R. Kandel, W. Alden Spencer, F. J. Brinley, Jr., and Wade H. Marshall of the Institute's Laboratory of Neurophysiology found that the pyramidal neurons of the hippocampus, unlike ventral horn cells in the spinal cord and pyramidal cells in the cortex, show depolarization after firing instead of hyperpolarization. These studies were made in the Ammon's Horn part of the hippocampus. The hippocampus, which is part of the temporal lobe structure of the brain, constitutes a phylogenetically old cortical field common to all mammals. This region of the brain is known to have a very low threshold for focal epileptiform discharge; when damaged in birth injury or other ways, petit mal epilepsy often results. The presence of depolarization instead of hyperpolarization after spontaneous and intracellularly evoked firing in hippocampal neurons is important since after-hyperpolarization would reduce the tendency to refire. After-hyperpolarization is characteristically present in single ventral horn neurons studied in the spinal cord and in the pyramidal neurons in the phylogenetically newer cortex of the brain.

This characteristic difference in the pyramidal cells of the hippocampus is a membrane property. While after-hyperpolarization is not an intrinsic property of this neuron, it can be produced by synchronous antidromic stimulation which presumably operates through the recurrent collaterals. It is not yet known if this effect is mediated through short axon interneurons. The investigators reported their findings at the meetings of the Federation of American Societies for Experimental Biology.

OTHER NEUROPHYSIOLOGICAL STUDIES

Two important studies by scientists in NIMH's Laboratory of Neurophysiology were reported at the American Physiological Society

meetings during 1959.

F. J. Brinley and Dr. E. R. Kandel have devised a very useful method of doing tracer work on the cerebral cortex. This method appears to be applicable to many chemicals but has so far been used only for K^{42} . The K^{42} is transferred into the cortex through the arachnoid-pia membrane system. The rate of release of the K^{42} is then followed as a function of various chemical agents or spreading cortical depression. It has been found, significantly, that gamma amino butyric acid causes only a small transient increase in K outflux. This strongly suggests that this drug does not act by depolarizing dendrites. It has also been observed that the surface negative phase of spreading cortical depression is accompanied by a very similarly shaped wave of K ion outflux from the cortex.

Dr. Felix Strumwasser has found that single cells can be excited extracellularly and has made an extensive study of this in frog brain. The stimulus is delivered through the recording electrode. This method permits identification of otherwise silent cells and gives previously unavailable information about the excitability characteristics of the cells.

BRAIN DAMAGE AND ABILITY TO GENERALIZE

NIMH grantees reported at the American Psychological Association meetings that children with

cerebral palsy suffer significant loss of ability to generalize stimuli.

Having learned to respond appropriately to a given stimulus, most people are then able to transfer this response to similar stimuli through a process that has been labeled "stimulus generalization". This useful capacity allows us to recognize as a chair an object whose design is novel; to behave in a useful manner in a situation, which, while new to us, contains elements similar to those we have

encountered before; and, indeed, is an essential capacity if the learnings we acquire in school are going to have any usefulness to us later in life.

NIMH grantees S. A. Mednick of Harvard University and Cynthia Wild of Yale University have demonstrated that children suffering from organic brain damage (in this case youngsters with cerebral palsy) differ significantly from nondamaged children of the same age and IQ in their capacity to generalize stimuli. The cerebral palsied children showed significantly less tendency to generalize stimuli in a laboratory study in which such generalization was normally frequent.

While other investigators have pointed out that the thinking of brain-damaged individuals is significantly more "concrete" than the thinking of normals, this study identifies that concrete quality as probably identical to the loss of stimulus generalization ability, thus tying these observations into the main stream of learning theory and research. Further, this identification makes it possible to draw upon the rich psychological research literature on stimulus generalization. From these studies it may be possible to find concrete suggestions as to ways of increasing these children's stimulus generalization capacity so as to improve methods of teaching them academic subjects. The authors point out the practical implications of this identification for selection of curriculum materials in special education programs for the brain damaged, and for providing further cues for the understanding of the physiological components of the learning process.

CORTICAL LESIONS IN MONKEYS

A study by an investigator now in the NIMH's Section on Animal Behavior, Laboratory of Psychology,

confirmed an hypothesis that performance on the critical flicker frequency test is not impaired by anterior frontal lesions of the brain but is impaired by inferotemporal and lateral occipital lesions.

These results confirm more generalized findings in man by scientists elsewhere and add both detail and conviction to the body of important new knowledge covering the relationship between brain structure and the capacity for complex perceptual responses and behavior.

The proposal by Halstead in 1947 that the frontal lobes are that portion of the brain most essential to "biological intelligence" has not been confirmed by ablation studies carried out on monkeys during the past ten years. These studies instead have shown that there is not a single but rather there are multiple transcortical gradients for "biological intelligence"; while frontal cortex is most important for certain kinds of complex behavior, other types of behavior are most adversely affected by lesions limited to posterior cortical regions.

The original study was performed at Institute of Living, Hartford, Connecticut. It will be reported in the Journal of Comparative and Physiological Psychology (in press). Dr. M. Mishkin, now of the NIMH research staff, and Dr. L. Weiskrantz, now of Cambridge University, England, studied 12 rhesus monkeys on whom anterior frontal, inferior temporal, and lateral occipital lesions had been performed. The critical flicker frequency test, a difficult test of visual discrimination, was used to study transcortical gradients of impairment. The results correlate specific impairment in this visual discrimination with inferotemporal and lateral occipital lesions and extend to more explicit brain mechanisms previous studies in man showing lasting depression in critical flicker frequency following damage to the occipital lobes.

LOCALIZATION OF GENITAL FUNCTION IN THE BRAIN

In studies aimed at further
elucidation of brain structures
involved in activities concerned

with preservation of the species, investigators Drs. Paul D. MacLean, Bryan W. Robinson, and Detlev W. Ploog, in NIMH's Laboratory of Neurophysiology, have conducted experiments on localization of the genital function in the brain. Using squirrel monkeys as the experimental animals, researchers applied electrical stimulation to selected sites in the limbic system, using penile erection as the end point of measurement, in a millimeter by millimeter exploration of the brain. It was found that stimulation in the medial preoptic region close to the septum results in penile erection with a latency of 6-7 seconds. These and related results appear to indicate that a portion of the limbic system involving parts of the septum, hippocampus, and cingulate gyrus is concerned with expressive and feeling states conducive to sociability and other activities directed toward preservation of the species.

All of the recognized emotions may be considered from the standpoint of self-preservation and preservation of the species. It is, therefore, extremely important to attempt to localize mechanisms in the central nervous system that are particularly concerned with the experience of emotion and its elaboration into behavior, and to attempt to ascertain how these mechanisms differ anatomically and functionally from other kinds of neural apparatus.

The limbic system refers to the lobe of the brain which forms a border around the brain stem. The limbic lobe, identified and described by Broca in 1878, contains most of the phylogenetically old cortex and is found as a common denominator in the brains of all mammals. The limbic cortex is structurally primitive compared with the neocortex. It shows essentially the same degree of development and organization throughout the mammalian series, suggesting that it functions at an animalistic level in both animal

and man. In contrast to the neocortex, the limbic cortex has strong reciprocating connections with the hypothalamus and other ancient structures of the brain stem important in integrating the performance of mechanisms involved in self-preservation and procreation.

Clinical and experimental work by neurophysiologists in the past have indicated that the frontotemporal portion of the limbic system fed by fibers from the amygdala is primarily concerned with self-preservation as it pertains to survival mechanisms involved in feeding and in the behavior required for obtaining food. Ablation studies have shown that bilateral excisions of this region interfere with the animal's capacity to eat properly and to protect itself.

In 1953, Dr. MacLean began to draw attention to other forms of behavior elicited by stimulation of parts of another circuit in the limbic system--the circuit from the medial forebrain bundle that converges with traffic from the medial olfactory tract and leads by way of the fornix and cingulum to the hippocampus and cingulate gyrus. The behavior elicited here might be interpreted as conducive to preservation of the species, rather than self-preservation. Electrical or local chemical stimulation of certain parts of the hippocampus, cingulate gyrus, and septum resulted in pleasure reactions, grooming behavior, and sexual manifestations, including penile erection.

Current investigations by Drs. MacLean, Robinson, and Ploog are concerned with millimeter by millimeter exploration of parts of the brain of male squirrel monkeys in order to localize structures responsible for penile erection. The findings, reported in Electroencephalography, permit the inference that the cingulate gyrus and hippocampus associated with the septal circuit are involved in activities related to preservation of the species. The close proximity to this region of the frontotemporal region of the limbic system (which is associated with self-preservation), the close relation of the oral and genital regions in cerebral organization, and the close connection of both to the olfactory sense, invite further speculation about the relationship of these neurophysiological structures to forms of behavior that are in the province of the social and psychological sciences.

MENTAL ILLNESS AND FAMILY RELATIONS

A number of studies of mental patients deal with family relationships and the role of the family as they affect the production and course of mental illness.

SOCIO-ENVIRONMENTAL INVESTIGATIONS

The Institute's Laboratory of Socio-Environmental Studies investigates the effects of social relationships and day-to-day habits of living on the individual's ability to function as a member of the family, the work group, and the community. One study by Dr. John A. Clausen, Chief of the Laboratory, concentrated on the impact of mental illness on the family. A preliminary report of this research, conducted with families from which either husband or wife was hospitalized for schizophrenia, reveals that these families frequently undergo a progression of disruptions and interpersonal conflicts well in advance of the patient's hospitalization.

For females, the marital role itself was most often the first to show a deficit, disruption coming through accusations of infidelity, moving out of the marital bed, or a substantial measure of psychological withdrawal. The role of wife as housekeeper was next most frequently impaired; housecleaning, cooking, doing the laundry became irregular or didn't get done. Only in one instance did the care of the children seem to be markedly impaired in the early stages.

By three months prior to hospitalization, half of the 14 female patients were performing almost none of their usual household tasks and two others were performing with substantial decrease in effectiveness or at markedly inappropriate times. Three of the ten male patients had likewise markedly diminished their performance of household tasks by three months prior to hospitalization and three others ceased to help at home during the last month. Their functions were, however, less critical to maintenance of the household. All but two of the male patients in the sample held their jobs and performed more or less adequately in them up to a month or less before they were hospitalized.

Almost all performance of essential life tasks was impaired at the end, though many of the women continued to care for their children and the men to meet the requirements of the job down to the last few days. Indeed, these seem to be the minimal role functions that must in general be sustained if the family is to go on at all. Extreme conflict may have been the pattern of marital interaction for months, but as long as the disturbed wife looked after her children or the disturbed husband managed to hold his job, some personal integration and some family integration was maintained. The findings were reported at the meetings of the American Sociological Society in Chicago and the Fourth World Congress of Sociology in Stresa, Italy.

FAMILY PSYCHOTHERAPY

Among the family studies carried out by the Institute's Clinical Investigations staff has been one involving the development, by Drs. Murray Bowen, Warren Brodey, and Robert Dysinger, of a method of family psychotherapy which is an integral part of a research study in which the family was regarded and treated as a unit. This approach was based on the hypothesis that the psychotic symptoms of one member are part of an active process involving the entire family group. The study was reported at the American Orthopsychiatric Association annual meeting at San Francisco.

The research plan involved having fathers, mothers, schizophrenic patients and normal siblings live together on the ward. The milieu was planned with the family unit in mind. The parents carried out many of the functions ordinarily assigned to nurses and occupational therapy was planned for the family unit.

With increasing experience with families in this "living together" situation, staff members were able to detach themselves from exclusive preoccupation with the problems of the individual and to see more of the over-all family problem. Once it was possible to attain this vantage point, it was possible to see family patterns that had previously been obscure.

The therapist saw the father, mother and psychotic patient at the same time, thus minimizing the development of emotional involvements characteristic of individual psychotherapy, and giving the therapist an opportunity to study the ways in which parents relate to the patient. Some patients in this study showed marked improvement, and life in these families has been established on a more mutually satisfying level. Continuing research in the patterns of family interaction will provide data important to greater understanding of both adaptive and non-adaptive behavior on the part of the individual members.

FATHER'S
ROLE

Significant patterns in disturbed familial relationships emerged from intensive research on a small number of fathers, mothers, and schizophrenic patients who were studied as a group and treated in family psychotherapy.

The study, an integral part of NIMH's clinical investigations into schizophrenia, included four families (father, mother, and severely impaired schizophrenic patient) who lived together on a psychiatric ward and participated in family psychotherapy for a period up to $2\frac{1}{2}$ years; and an additional six families (father, mother, and overtly psychotic schizophrenic patient) treated in outpatient family therapy for a period of up to 2 years. The function of the father, as he participates in the day-to-day life of the family, was studied intensively.

In all ten families there was a striking emotional distance between the parents. Some of the parents maintained a positive but formal and very controlled distance with few overt differences; they look at their marriage as "ideal". Other parents were aware of differences but constantly avoided touchy points. At the other end of the scale were parents who could not remain long in physical proximity without open arguments and disagreements.

The fathers and mothers appeared equally immature. The families were incapable of many decisions that are routine for other families. The greatest conflicts among the parents were in their convictions about proper treatment of the patient.

The fathers and mothers were separated from each other by an emotional barrier which in some ways has the characteristics of an "emotional divorce". Either father or mother was able to have a close relationship with the patient, if the other parent permitted. The usual pattern was one in which the mother had a close relationship with the patient and the father was excluded, or permitted himself to be excluded. The patient's function was similar to that of an unsuccessful mediator of the emotional differences between the parents. These disturbed patterns changed with therapy.

The study was reported by Drs. Murray Bowen and Robert H. Dysinger and Miss Betty Basamania in the American Journal of Psychiatry.

RIGID FAMILY EXPECTATIONS

Clinical observations by NIMH
psychiatrists Drs. Irving M.
Ryckoff, Juliana Day and Lyman

C. Wynne, have led them to hypothesize that family structures which assign rigidly defined patterns of behavior (social roles) to their members may precipitate psychotic behavior. This can occur when the family imposes upon the individual a role which he cannot maintain and which denies him opportunities to experiment with other roles as a way of developing individuality within the family unit. The denial of freedom to experiment with other roles may reflect severe anxiety within the family, and any deviation from the expected role may be resisted because of the anxiety-producing effects of such deviation. The investigators reported on their work at the meetings of the American Psychiatric Association. They particularly stress the resulting inability of the individual to select or develop new roles, and suggest that the lack of adaptability thus produced may leave the individual unable to cope with new experiences by other than a diffusion of his identity such as is found in acute schizophrenic episodes.

FAMILIES IN TREATMENT

In a significant contribution toward the understanding of the pathogenic family, NIMH grantee

Dr. Erika Chance developed new research methods for studying the interpersonal relations between patient and therapist, for measuring the progress of therapy at any stage, and for evaluating therapeutic techniques and results.

She centered her attention on such relevant issues as: Do children who attend child guidance clinics have immature parents whose dependency needs compete with those of the children? Do fathers and mothers have neurotic needs which complement each other? What kinds of relationships are most conflict-laden for the family? Do patients of optimistic therapists show more change than those of pessimistic therapists? How do mutual expectations of therapists and patients affect the course of treatment?

The major thesis of her book is that a combination of clinical and research descriptions of families in treatment contributes more to our understanding than the separate use of either. Families in Treatment (From the Viewpoint of the Patient, the Clinician, and the Researcher), Basic Books Inc., N. Y., 1959, is based on a five-year study and is concerned primarily with the methods by which the difference in attitudes of clinicians and researchers can lead to fruitful collaboration between the two specialties. These techniques can be used to expand our understanding of the nature, process, and outcomes of neurotic interaction in the family.

HEALTH INSURANCE FOR PSYCHIATRIC ILLNESS

With the aid of an NIMH grant, Group Health Insurance, Inc., a nonprofit medical service insurance plan, is covering a selected group of their subscribers with comprehensive health insurance including psychiatric illness.

Support of the three-year experiment will make these additional services available without extra premium charge. Information will be gathered on the use of the psychiatric treatment services in relation to the age, sex, marital status, medical history, occupation, income and education of persons covered. Purpose of the study is to determine whether psychiatric care is an insurable risk. It is postulated also that coverage for mental illness will serve to encourage people to seek help promptly and thus prevent serious long-term mental illness.

During the early years of medical care insurance, nervous and mental disorders were traditionally excluded from coverage. Factors contributing to this situation included: the assumption that the State was responsible for these people; the fact that there was no actuarial basis for estimating costs; the assumption that any effective treatment had to be of long duration making it highly expensive;

and the fact that professional personnel and treatment facilities were not available. With increasing awareness of the prevalence and social cost of psychiatric disorders and with increased knowledge and new techniques for effective short-term treatment, there is great interest in preventive care.

Under the Group Health Insurance, Inc., plan subscribers will be covered for short-term treatment of mental illness, including individual and group therapy, electroshock and anesthesia, psychological testing and 30 days of in-hospital treatment. Cost of drugs is not included under the plan. Patients will pay \$5 for each individual psychiatric therapy session and \$1 for each group therapy session. The health insurance policy covers fees for psychological testing up to \$45.00.

THE MENTAL HOSPITAL

The mental hospital itself has become the subject of much study during the past few years. The decline in the hospital population with the advent of drugs and the success of new therapeutic techniques within the hospital have raised new hope as well as new questions about how the hospital can become a better treatment tool.

During 1959, nine States passed legislation bringing them into the Interstate Compact on Mental Health which permits waiving of residency requirements in determining where mental health services shall be provided for a patient. This makes it possible to provide mental health services promptly when an individual becomes ill and is not in his home State. Legislative action during 1959 brings the total number of participating members of the Interstate Compact on Mental Health to 21.

DECLINE IN MENTAL HOSPITAL POPULATIONS

Data from NIMH's Biometrics
Branch indicate that resident
mental hospital populations

continued to decline for the fourth consecutive year between 1958 and 1959. The 542,721 patients resident in 277 public mental hospitals at the end of fiscal year 1959 represented a decrease of 2,142 from the corresponding number at the end of fiscal year 1957. This decrease of 0.4% compares with a decrease of 0.7% between 1957 and 1958, 0.5% between 1956 and 1957, and 1.3% between 1955 and 1956. These figures incorporate the effects not only of the number of releases but also of the number dying and the total admissions occurring within the year. Data indicate that total admissions increased 4.3%, 4.8%, 7.7%, and 6.5% in 1956, 1957, 1958, and 1959, respectively over the previous year; the corresponding percent changes in the number of deaths was 8.7%, -2.9%, 9.5%, and -3.3%.

CHANGING PATTERNS OF HOSPITALIZATION

Careful and detailed analyses are being made to determine fully the meaning and significance of the changing patterns in the movement of the mentally ill in and out of the public mental hospitals of the nation. One such study, reported in Public Health Monograph No. 58, Patterns of Retention, Release, and Death of First Admissions to State Mental Hospitals (1959), describes the experience during the first 12 months of hospitalization of patients admitted to 11 State hospital systems in 1954. The objectives of this analysis, carried out by 11 States in the Model Reporting Area, in cooperation with the Biometrics Branch, NIMH, were: To present data on patient flow following first admission in different State mental hospital systems; to emphasize the ways in which basic data differ among the States and the resulting problems in comparing and interpreting them; to highlight the technical problems involved in such an undertaking; and to point up the type of material, methodology and special studies needed to make interstate comparisons meaningful. The project also points up the importance of studying the mental hospital in relation to the entire range of psychiatric facilities in each State.

SUPPORT FOR HOSPITAL IMPROVEMENT

The Institute's Mental Health Project Grants Program, initiated in 1957 under authority of Title V

P.L. 911, supports projects aimed at new and improved methods for the care, treatment, and rehabilitation of the mentally ill. Although most of these projects are still in process, some have progressed far enough to permit preliminary assessment. One such project, being conducted by Dr. Richard Sanders and associates at the Philadelphia State Hospital, is aimed at establishing and evaluating an intensive psychosocial treatment program for chronic psychiatric patients. A therapeutic milieu has been created for patients originally in a custodial setting. A home-like atmosphere has been introduced, featuring increased personal privacy, decreased regimentation, and increased self-care. There is intensive inservice staff training at all echelons, including both professional and nonprofessional personnel. A broad program of patient activities has been introduced (education, work, music, recreation, group therapy, patient self-government), with emphasis on increased interaction among patients rather than on mere acquisition of skill or passive compliance with the schedule. The patients have greater freedom of movement. They have keys to their own building and rooms, and they are allowed to visit towns and to hold down extramural part-time jobs.

Preliminary indications are that such measures are effective in the rehabilitation of heretofore "chronic" patients. Results to date on a pilot group of 65 patients, whose average length

of hospitalization prior to the project was 13 years, show that 55 percent were able to leave the hospital directly from the program and an additional 8 percent were able to leave later. About 85 percent of those leaving were still living in the community at the time of first follow-up 2 to 15 months later, and of these about 38 percent were working on jobs. Of the 36 patients who were able to leave the hospital under this program, only two were described as making a "poor" adjustment on the outside. Definite improvements in employee morale are also reported. The extension and applicability of such intensive and comprehensive measures holds much promise not only for acutely ill patients but also for chronically ill patients, who have for so long comprised a major factor in the high costs of public mental hospitals and who have been a source of much discouragement.

DRUG THERAPY AND MILIEU CHANGE

The Institute's Laboratory of Socio-Environmental Studies has focused an important part of

its investigations on those elements in the mental hospital environment which prove to be therapeutic. The investigations are concentrated on the reactions of staff to new therapeutic techniques, as well as on the results of experimental attempts to increase social interaction among patients.

One such study, in which Dr. Erwin L. Linn of NIMH's Laboratory of Socio-Environmental Studies examined release rates among patients admitted to Saint Elizabeths Hospital for the first time during the period January 1, 1953 through August 31, 1956, revealed that the patients hospitalized during 1955-1956, whether or not they were treated with chlorpromazine or reserpine, were more likely to be released to the community than patients hospitalized before the use of drugs in 1953. During the years studied, there were no changes in diagnostic categories, symptomatology, or previous social histories of patients entering the hospital. Dr. Linn, reporting his study in the A.M.A. Archives of Neurology and Psychiatry, theorizes that the increasingly optimistic expectation of staff that patients would recover, perhaps due to successful treatment of chronic patients, had increased the probability that all patients, whether or not treated with drugs, would recover. In a further analysis of these data reported in the American Journal of Sociology, Dr. Linn found that the most striking increases in release rates were among unmarried patients who are from the working class. There were increases in release rates for patients from all socio-economic groups, but the release rates for working-class patients, which had been quite low, showed the most striking increases. Since the unmarried working class group had previously constituted the bulk of patients who became chronic, this change in release rates will have a strong influence on the social characteristics of the resident population in mental hospitals.

CLINICAL NEUROPHARMACOLOGY
RESEARCH CENTER

During the past year, a comparative drug study was planned and carried out in six wards of

Saint Elizabeths Hospital's William A. White Service. This is the building which houses the Clinical Neuropharmacology Research Center, a joint project of NIMH and the Hospital.

The four principal aims of the study were to: (1) test the feasibility of applying, in a busy mental hospital ward, certain administrative procedures designed to make the most economical use of ward personnel; (2) determine ways whereby such a study could develop staff members' therapeutic skills; (3) test the usefulness and reliability of various clinical research instruments; and (4) determine the effect of clinical research programs on staff attitudes. The study concentrated on the systematic introduction of group nursing methods and use of psychoactive drugs. Thirteen different research instruments (rating scales) were employed during the course of this investigation.

The methods used have proved feasible and economical of staff time. The introduction of group nursing methods as part of the drug trial favored the development of similar group techniques outside the framework of the formal trial; this, in turn, has favorably affected treatment procedures used within the Service as a whole. Experience has been gained in the training of staff. With the active participation and encouragement of the Hospital, the pilot study is now to be extended to some other Services. It has also been clearly shown that a rather complex research design can be pursued in a typical State Hospital setting, despite limitations of budget and staff, and lack of optimal physical facilities.

Another study being conducted by the Center is attempting to define the factors making for chronic hospitalization, with special reference to an analysis of so-called "dependency" factors in the patient, and the possible (though unintended) interaction between the needs of the over-dependent patient and an over-protective regimen in a chronic hospital ward. A method for the objective (and visual) recording of the relative social interaction of the individual patient with other patients and staff is being developed. This should be of particular interest in any study of devices or procedures (including drugs) designed to render the patient more accessible to the milieu within which he functions. A pilot study of the effect of group psychotherapy in facilitating the chronic patient's transition from the Hospital to the community is being initiated.

In addition, a number of basic metabolic and other biochemical studies are being carried out by the Center.

CHILDHOOD STUDIES

Much of the research conducted and supported by the National Institute of Mental Health is concentrated on basic studies of child development and of environmental conditions during childhood which influence normal personality development and the development of pathology.

SOCIOLOGICAL
INVESTIGATIONS

Work in this area conducted by the Institute's Laboratory of Socio-Environmental Studies is

concentrated on the influence of family relations and peer relations on child development. One series of studies deals with the influence of mothers' work outside the home on the mother-child relationship and the differences in family relationships between families where the mother works and where she does not work. These studies are also concerned with persistence and change in patterns of mother-child relationships from early childhood through adolescence. The Laboratory is studying methods of eliciting data through interviews and observations in order to obtain the most valid picture of family relationships.

INFLUENCE OF
SOCIAL CLASS

In their investigations of the relationship between social class and parental values and the

exercise of parental authority, Dr. Melvin L. Kohn of the Laboratory of Socio-Environmental Studies and Dr. John A. Clausen, Chief of that Laboratory, found significant differences between the ways in which working class and middle class parents attempt to shape the character of their children. Working-class parents tended to emphasize the importance of obedience, conformity and what were taken to be "middle class values" a generation ago. Middle-class parents tended to stress the development of responsibility, and the importance of happiness and creativity. The parents all had a child aged 10-11 and in the fifth grade at school.

The investigators also found that the conditions under which middle- and working-class parents punish their pre-adolescent children physically, or refrain from doing so, appear to be quite different. Working-class parents are more likely to respond in terms of the immediate consequences of the child's actions, middle-class parents in terms of their interpretation of the child's intent in acting as he does. This reflects differences in parents' values: Working-class parents value for their children qualities that assure respectability; desirable behavior consists essentially of not violating prescriptions. Middle-class parents value the child's development

of internalized standards of conduct; desirable behavior consists essentially of acting according to the dictates of one's own principles. The first necessarily focuses on the act itself, the second on the actor's intent.

In a related study, it was found that the father-son relationship is much closer and warmer in the middle-class family than it is in the working class family. All of the above differential social class factors are being studied in terms of the relationship between social status and rates of mental illness.

The investigators have reported their findings in the American Journal of Sociology and the American Sociological Review.

CHILD DEVELOPMENT

The Institute's Section on Child Development is studying the interactions between genetic and environmental factors in early behavior development, as well as the nature of learning in the early stage of human development. One aspect of this program is to revise and standardize an improved scale for measuring and scoring mental, motor, and personality development in infants. Such a scale can then be used as a tool in evaluating the effects of learning experiments in relation to the infant's degree of maturity, as well as for a general diagnostic tool. One study is focused on the drives and reaction tendencies of normal infants (e.g., curiosity and the need for visual and other stimulation), and their relation to infants' learning and personality formation. Another study is being made of the behavior of infants reared in such different environments as an institution and a "middle class" American home, with detailed observations of the actual environments experienced by the infants.

Dr. Nancy Bayley, Chief of the Section on Child Development, served as chairman for the 25th Anniversary Meeting of the Society for Research in Child Development held at NIH in March 1959. Approximately 200 scientists from universities, hospitals, and government agencies attended the 3-day conference devoted to discussion of current research in the field of child development and the prognosis for the next 25 years.

BIOSOCIAL GROWTH STUDIES

The NIMH has initiated a new series of studies on infants and young children, concentrated on emotional, biological, and cultural factors as they affect personality development. The development of methods for identifying family interaction patterns which tend to facilitate the personality development of the child as opposed to those which

hinder such development will have public health value. In addition, the basic research done on infant personality development may have implications for timing and type of preventive action. Specific short-term studies which attempt to relate infant personality variables to the family situation will form the basis for a later longitudinal research program which will encompass the prenatal family environment as well as the formation of early childhood extra-familial relationships, and will evaluate the precursors and the sequelae of events that occur during infancy.

PREDICTION OF GOOD AND POOR ADJUSTMENT

In a study originating in 1949, the Institute of Child Welfare of the University of Minnesota conducted a survey of the mental health of children in Nobles County, Minnesota. Financial assistance was provided from 1953 to 1959 by the National Institute of Mental Health. During this time, an extensive series of investigations were carried out, aimed at devising and testing instruments which might be helpful in predicting mental health over a period of time. The investigators attempted the difficult task of extending to the areas of personality, adjustment, and mental health the development of measurement procedures similar to those which psychologists have devised for predicting the quality of an individual's performance in a task or on the job. Reliable predictions of the future personality development and behavior of children would have great utility for planning educational programs to capitalize on the child's capacities, and for identifying children who are in the early stages of mental and emotional disorders and in need of prompt treatment.

One of the important findings in this study was that teachers' ratings of children and the scores the children made on various intelligence tests gave a better indication of the children's later adjustment than did personality tests. It appeared to be easier to predict correctly whether a child would make an outstanding adjustment than whether he would make a poor adjustment. Among predictions of poor adjustment, the greatest number of correct predictions concerned the children who would drop out of school; the next greatest number of correct predictions concerned those who would become delinquent.

Related studies forming part of the over-all Nobles County project supported by NIMH revealed considerable agreement between men and women teachers in the criteria used to characterize best and poorest adjusted high school youth, with intellectual and achievement variables playing an important role. The criteria used significantly more by men teachers are maturity, good judgment, dependability, trustworthiness, lack of self-consciousness,

and being secure as a person (the "emotional-personality" items). The criteria employed more characteristically by women teachers are humility and modesty (the "character-control" items).

It was also found that, when compared with poorly adjusted children, children who are well adjusted consistently give more pleasantly rather than unpleasantly toned responses, and express likes rather than dislikes with respect to particular activities.

The investigators reported their findings in the Journal of Educational Psychology and Education, and at the Symposium on Child Development held at the University of Texas.

JUVENILE DELINQUENCY

The NIMH and the Children's Bureau have prepared, at the request of Congress, a report

which attempts to answer the question: What can and should be done to control juvenile delinquency? The report includes both substantive and fiscal proposals for action considered to be necessary and desirable in attacking the problems of juvenile delinquency in the United States. Juvenile delinquency is the major mental health problem among boys in the United States. Thorough study of the whole problem will bring valuable information, in addition, about child development, particularly development of pathology. The Institute is interested in prevention and treatment of juvenile delinquency, as well as in research in this field and training of personnel to deal with the problem. Several agencies, with support from the Institute, have designed community-wide delinquency control projects to test the effectiveness of present techniques and recommended new services that appear to hold promise for the prevention of delinquency. The Institute is already partially supporting such programs in several urban centers. Recently large-scale support was given to initiate a major demonstration project and field experiment for delinquency control.

STUDIES ON AGING

The Institute's program of research on aging continues its progress in learning more about the process of aging itself as part of human development and the characteristics which are attributable to normal or abnormal aspects of the process.

HANDBOOK ON AGING

One of the most notable advances during 1959 was the editing and publication, by members of the

Institute's Section on Aging, of the Handbook of Aging and the Individual: Psychological and Biological Aspects (December 1959,

University of Chicago Press). The absence of a published systematic and comprehensive survey of the literature has been a handicap to the planning of research on the behavioral aspects of aging. The new Handbook, edited by Dr. James E. Birren, Chief of the Institute's Section on Aging, is an authoritative technical summary of the scientific and professional literature on the psychological and social aspects of human aging. Its 24 chapters, prepared by recognized authorities, range from the most detailed to the broader aspects, from the cell to the whole organism, but the primary emphasis is on age changes in human behavior and the bases for these changes in both biological and social processes. The book is divided into 4 parts: Foundations of Research on Aging; Biological Bases of Aging; Aging and Environmental Settings; and Psychological Characteristics of Aging. Five of the 24 chapters were written by Drs. Birren, William Bondareff, Alfred A. Weiss, Edward A. Jerome, and Jack Botwinick of NIMH's Section on Aging. It is expected that the Handbook will facilitate systematic research and the development of undergraduate and graduate courses of instruction in the field of aging.

INTERDISCIPLINARY STUDY OF AGING

Data obtained from the Institute's interdisciplinary study of aging in healthy elderly men are

being prepared for publication in a monograph tentatively entitled Human Aging: Biological and Behavioral Aspects. NIMH staff specialists involved in the study have contributed the various chapters to this research report on physiological, physical, psychiatric, psychological, and social findings. The entire volume is being edited by an interdisciplinary group of NIMH scientists who participated in the research.

Preliminary analysis of the data indicates that healthy older men (all subjects in the study were over 65, the average age being 72) are not very different from healthy younger men, and that illness rather than age often accounts for the typical picture one has of a group of older individuals. Physiological measurements, such as cerebral blood flow and cerebral oxygen consumption, are not significantly different for healthy older men than they are for younger men. When the group was subdivided into 27 who were healthy and 20 with a subclinical though non-overtly limiting condition (e.g., mild hypertension) certain psychological differences were noted that could be related to the level of physiological functioning. However, both subgroups differed significantly from younger men on certain psychological measurements, indicating that, although health alone seems to be a factor in the physiological differences between young and old, normal "aging" as well as health is important in psychological differences between the two age groups.

Dr. Sokoloff and his associates in the Laboratory of Clinical Science demonstrated the maintenance of normal cerebral circulation and cerebral oxygen consumption in this group of elderly, healthy males, showing that reductions in these functions are not a necessary concomitant of aging. He has also adduced additional evidence suggesting the importance of cerebral circulatory insufficiency as a primary factor in certain senile deteriorations of mental function.

PSYCHOLOGICAL CAPACITIES AND BEHAVIOR

A number of studies conducted in the Institute's Section on Aging are concentrated on the measurement of psychological capacities of men and women in different age groups. In instances where reliable differences in behavior with age are found, an effort is being made to understand the nature of the difference, the reason for its occurrence, whether it is reversible or irreversible, whether it has a biological antecedent or is the result of learning and habit. These studies are being conducted in three major areas: thinking and problem solving, psychomotor skills, and perception. Work in the area of thinking and problem solving is at an early stage; the Logical Analysis Device of the Psychological Corporation is being used to study age differences in how people attack the various elements of solving a problem and in general how people approach problem solving situations. Work has gone on for a longer time in the areas of psychomotor skills and perception and some interesting results have already been published.

In a comparison of abilities of younger and older subjects to learn and perform a graded series of card-sorting tasks, reported by Jack Botwinick, Ph.D., Joseph S. Robbin, and Joseph F. Brinley, staff members of NIMH's Laboratory of Psychology found that the older group did relatively poorer on the task requiring the most mental manipulation and perceptual searching. Poor performance was also seen when age differences in motor or movement performance were examined. As the task became more difficult, both age groups slowed in their performances up to a critical point in complexity of operations required. At this critical point, the increased difficulty and complexity of the task made for disproportionately poorer performance in the elderly. The rate of improvement with practice within each difficulty level was the same for both age groups. The study was reported at the 1959 meeting of the American Psychological Association.

Another study by Brinley, Robbin, and Botwinick indicated that when conditions facilitate accurate anticipation of the time that a stimulus will occur, increased motivation will improve the simple reaction-times of older subjects more than that of

younger subjects. When conditions do not facilitate accurate anticipation, increased motivation will improve the reaction-times of older and younger subjects to about the same extent. The regularity or irregularity of the preparatory interval was a key factor that made for the accuracy of anticipation. When the time interval between the warning signal and the stimulus was kept constant, i.e. was regular, the accuracy of anticipation was higher than when the interval was varied.

Earlier studies conducted by the same investigators were aimed at answering the question as to whether motivation, or lack of it, accounted for the slowing in reaction time that occurs with age. First, median reaction times were secured for each subject. Then, each subject was told he would be given a mild electric shock each time he fell below his own median. The shock motivator decreased reaction time considerably for both young and old subjects, but the extent of improvement of both age groups was approximately the same. The investigators concluded that if motivation were a factor, it operated equally for both age groups and the longer reaction time of the elderly cannot be attributed to a differential in motivation.

In the present study, reported at the American Psychological Association meeting, 24 subjects aged 65-81 years and 27 subjects aged 19-31 years were given the same test under two different sets of conditions. Under the first condition, the interval between the warning signal (a light) and the stimulus (a tone) was irregular, varying at random from 1 to 6 and from 1 to 25 seconds. The results here were the same as in the previous study; both young and old subjects improved performance to approximately the same extent. In the second condition, the preparatory time interval was kept constant over a substantial number of trials. Under this condition, the performance of the older subjects improved even more than that of the younger subjects. It was concluded, therefore, that conditions of motivation and anticipation interact and must be taken into consideration in the analyses of age changes in speed of response.

In a study of the differential effects of aging on verbal abilities, Dr. Klaus F. Riegel, Visiting Scientist in NIMH's Laboratory of Psychology, found that the performance of older individuals decreases as the number of possible associations (response words) to a given test word increases. Five verbal tests were developed (Synonyms, Antonyms, Selections, Classifications and Analogies) and given to 74 persons above 65 years of age and to 56 students from professional schools with an average age of 18.6 years. Dr. Riegel's study, reported at the American Psychological Association meeting, was designed to test the hypothesis that associations between linguistic-logically

related words became strengthened with life experience. Linguistic-logical associations play an especially important role for the solutions of the Synonym Test, because there exists only a very limited number of possible associations, namely synonyms, to a given word in any language. The older group did slightly better than the younger group on this test. In the Analogy Test, a specific relationship has to be recognized and this relationship has to be generalized and transferred to a different section of the association space. In this test, the performance of the older group was only slightly over half of that for the younger group.

A comparative factor analytic study of the scores made by different age groups on the Hamburg-Wechsler Intelligence Test for Adults (HAWIE) revealed qualitative changes in the verbal-performance factor with age. Working with test standardization data on approximately 1,000 subjects divided into four age groups (20-34, 35-49, 50-64, and 65-75 and over), Dr. Ruth M. Riegel, Visiting Scientist in NIMH's Laboratory of Psychology found that the general factor of intelligence, common to all 10 subtests in the HAWIE, remained constant with age, whereas the general verbal comprehension factor increased with age. The younger individuals' performance in the verbal tasks is more determined by general information (that is, recently learned material), whereas the performance of older individuals was more determined by general comprehension (the material of daily experience). Dr. Ruth Riegel's study which is the first to concentrate on data from the HAWIE, shows, incidentally, that there are no significant cultural differences between performance of American subjects on the W-B test and performance of German subjects on HAWIE. Dr. Ruth Riegel presented her results at the American Psychological Association meeting.

Analysis of psychological measurements made on a group of relatively healthy elderly men has made possible the construction of a single critical score which can be used to distinguish individuals without physical disease from those with disease. This study, conducted by Dr. James E. Birren, Dr. Jack Botwinick, Dr. Alfred A. Weiss, and Donald F. Morrison in NIMH's Laboratory of Psychology, utilized some 150-200 psychological measurements that had been made during the large NIMH interdisciplinary study of aging involving contributions from the biological, the psychological, and the social sciences.

Using data from tests which measured, among other factors, reaction time, perception, and verbal and motor learning of a group of elderly subjects (normal volunteers, aged 65 years and over) under a variety of conditions, the investigators made a series of intercorrelations from which 32 independent scores were extracted. These 32 scores were subjected to an analysis to extract the "principal components" that were measured by scores.

As a result of this analysis, each subject was given over-all scores on four or five major components, and these scores were combined into a single score for each person. The resultant score proved to be effective in distinguishing between subjects with no physical disease and those with disease, even though both groups were above average in health for their age. The investigators reported this work at the meeting of the American Psychological Association.

ALCOHOLISM

COOPERATIVE COMMISSION ESTABLISHED

Alcoholism, a public health problem of major proportions in the United States, is the subject of a major study

supported by the Institute. A million dollar grant has been made to the North American Association on Alcoholism Programs (an organization of alcoholism program administrators in the United States and Canada) for the purpose of establishing an independent Cooperative Commission on the Study of Alcoholism. This Commission will re-examine the whole problem of alcoholism in the United States and Canada and recommend future policy and action. Up until now only piecemeal, administratively uncoordinated efforts have been made against alcoholism. While these efforts have resulted in wide experience and much literature, there has been no single body to review and evaluate what has been done and to map a plan of action. For the first time, a unified and impartial approach will be made to the urgent and costly problem for which we have not yet found solutions. Among the pressing problems to which the Commission will address itself are:

1. How alcoholism is affected by the activities of institutions such as State public health and mental health departments, the courts, and law enforcement agencies.
2. The obstacles to effective action against alcoholism.
3. The supply of and demand for personnel to work in the field of alcoholism, training for such personnel, and recruitment problems.
4. How to extend and apply, in other parts of the country, alcoholism programs which have proved successful in one locality.

STRENGTHENING
ALCOHOLISM
PROGRAMS

Since 1955, the Community Services Branch of the National Institute of Mental Health has expanded its technical and consultative

services to the States through the Technical Assistance Projects program. This technique has made it possible, when a State requests help on a specific problem, for the Institute to support workshops or conferences which bring top experts in the problem field to the State to work with personnel there. This Technical Assistance Projects program has become an effective mechanism for strengthening community mental health activities, coordinating mental health programs, and bringing expert knowledge on specific subjects to people working on State problems. The impact of these projects is not limited to the States in which they originate since reports are published and distributed widely throughout the country.

During 1959 Technical Assistance Projects on alcoholism were held in 10 States on such subjects as community resources for the rehabilitation of the alcoholic, alcoholism as a mental health problem in business and industry, mental health aspects of alcohol education, and the family-centered approach to alcoholism. The conclusions reached by these institutes stressed the importance of approaching the subject of alcoholism on a community basis and affirmed the efficacy of concerted effort in doing something about the problem.

One of these institutes, devoted to the subject of the role of religious counseling in the rehabilitation of the alcoholic, explored the responsibility of the clergyman in working with alcoholics and discussed such factors as early detection, counseling techniques, referral, motivation, family implications, medical and psychiatric considerations, coordination with other services in the community, and the function of the clergyman in assisting the alcoholic in his reintegration into the community after treatment.

COUNTY HEALTH DEPARTMENT
ALCOHOLISM PROJECT

A collaborative project sponsored by the Mental Health Study Center, NIMH, and the Prince

Georges (Maryland) County Health Department is designed to demonstrate, over a four-year period, a public health approach to problems of alcoholism in the family. This demonstration will focus on the effectiveness of integrating an alcoholism program into the complex of public health and mental health planning and activities of a community health department.

Alcoholics participating in the demonstration will be referred by private practitioners, the courts, the police, hospitals, and social agencies in the County. Services for the alcoholics and their families will be provided by the Prince Georges General Hospital and the Mental Health Division of the Prince Georges County Health Department.

Research rather than treatment is the primary goal of the demonstration. A study will be made of family problems created when one member is an alcoholic, of how many alcoholics will accept services designed to help them, the kinds of services they will accept, what community agencies bear the greatest burden in relation to alcoholism, which agencies will work with alcoholics and how to gain acceptance from those agencies and groups that have resisted dealing with the problem of alcoholism.

STUDY IN A TRI-ETHNIC COMMUNITY

A Colorado community of 400 families composed of three ethnic groups, Ute Indians, Spanish-Americans, and Anglo-Americans, is the laboratory for a five-year project aimed at obtaining information on the entire range of drinking behavior, as well as other data on the mental health of the community. Three major problems will be explored in this research under the direction of Dr. Omer C. Stewart, Chairman of the Department of Anthropology, University of Colorado. The first is to determine the prevalence and variety of patterns of maladjustment among the three ethnic groups. The second is to determine to what extent variables in group attitudes, values, and social structure and other cultural patterns correlate with differences in the mental health of the groups. The third is to find to what extent any prevalent evidences of maladjustment, including drinking behavior, can be understood because of personality characteristics such as dependency and level of aspiration, and cultural forces such as adherence to traditional beliefs and ease of social intermingling among the three cultures or within a single cultural group. Educators, public health officials and social workers attempting to deal with alcoholism as a health problem in all cross-cultural contexts, but especially among Indians where the problem is of particular concern, will find the results of this phase of the study of practical importance.

BASIC PSYCHOLOGICAL STUDIES

The NIMH's Addiction Research Center at Lexington, Kentucky, has recently been extending its studies of the physiological and psychological aspects of addicting drugs to include work with alcohol. Psychological profiles have been collected on 200 narcotic drug addicts, 200 criminals who are neither alcoholics nor drug addicts, and on 200 alcoholics. Preliminary analysis of

data shows similarities in the profiles of these groups. An inventory of questions designed to reveal the habits and attitudes of alcoholics has been announced by the Lexington Hospital staff and administered to 350 alcoholics. A similar inventory will be used to ascertain the habits and attitudes of opiate addicts, so that a comparison can be made.

The Addiction Research Center has done research for many years on the physiological phenomena of tolerance to opiates in man. It is now working on the development of a suitable instrument to test the subjective effects induced by such drugs. It is planned to extend these types of inquiries to the subject of alcoholism.

COMMUNITY MENTAL HEALTH SERVICES

State mental health programs continue to expand and develop at an increasing rate. Institute cooperation and technical and professional assistance have played an increasingly large part in this growth.

Federal, State and local funds budgeted by the States for community mental health services reached a new peak of \$64.8 million in fiscal year 1959 — a 20% increase (\$10.8 million) over the previous year. Federal grants-in-aid of \$4 million represented only 6% of the total funds budgeted. State and local community mental health services have continued to expand on many fronts. About \$9 million of the funds are budgeted for the expansion of clinical and local mental health services. The remainder of the increased funds are to be used to expand State-level staff, research and training — all areas in which there is great need to strengthen present programs. Although there has been a rapid growth in the number of clinics since 1946, when the Mental Health Act was passed, lack of such services in rural areas throughout the United States still continues to be a major problem.

During 1959, surveys of mental health services in West Virginia, Colorado, and Nevada were made by NIMH staff at the request of the governors of the three States. In the District of Columbia, at the request of the Director of Public Health, NIMH provided consultation for a survey by D. C. Department of Health staff. In each instance the purpose of these surveys was to improve existing mental health facilities and programs. The District survey resulted among other things in the establishment of a Bureau of Mental Health in the D. C. Department of Health.

During April 1959, the Institute held the second of a series of orientation conferences for State-level personnel. These meetings provide an opportunity for State people responsible for mental health program planning to share new ideas, and to discuss

present programs and ways of strengthening and expanding them. Sixty-five representatives from selected States attended the meeting.

TECHNICAL ASSISTANCE PROJECTS

In 1955 the Community Services Branch of NIMH evolved the idea of providing support for workshops and conferences held in

a specific State on a specific subject directly related to the development of their mental health program as an additional way of meeting the many requests from the States for technical and consultative assistance. This Technical Assistance Projects program has become an effective mechanism for strengthening community mental health programs, coordinating mental health activities and bringing to people working on State problems expert knowledge on specific subjects. To date, 27 technical assistance projects have been completed.

SUICIDE REFERRAL SERVICE

A center for community-wide referrals of suicidal persons has been established in Los Angeles. This five-year

project supported by Mental Health Project Grant funds utilizes community agencies in obtaining referrals of suicidal patients, and both public and private treatment facilities in handling the patients. The center staff includes psychiatrists, psychologists, and social workers. The broad purpose of this demonstration project is to obtain more precise and scientific information on suicide.

The center is recognized as the place where the most comprehensive research on suicide is being conducted both in the United States and on a world-wide basis. This particular study has grown out of a number of previous studies some of which were also supported by NIMH. The two principal investigators, Edwin S. Shneidman, Ph.D., Department of Psychology, University of Southern California, and Norman L. Farberow, Ph.D., Clinical Psychologist, Veterans Administration Mental Hygiene Clinic, Los Angeles, have collected complete records on all suicides or attempted suicides in Los Angeles County during the last 10 years. Their book Clues to Suicide published in 1957 is one definitive product of earlier research which has provided the broad foundation for the present demonstration project. The other unique aspect of this program, particularly from a public health point of view, is the complete and firm support of the community. The Coroner's Office, the Los Angeles County Medical Society, the Los Angeles Osteopathic Society and the police, together with other pertinent community agencies, are cooperating fully with the investigators.

Referrals of suicidal patients to the center are made by such agencies as the Los Angeles Emergency Hospital, physicians in Los Angeles County, Los Angeles Police, and social agencies. At the center these individuals are interviewed and tested. Based on the diagnosis made, they are then referred for treatment to the most appropriate resource in the community -- a general hospital, a sanitarium, a private psychiatrist. Arrangements have been made whereby the treatment resource reports back to the center on care provided and the patients' response.

The purposes of this demonstration project are: To develop, test, and refine hypotheses about the primary, sustaining and precipitating causes of and appropriate treatment for suicidal patients; to obtain psychiatric, psychological and social work data from suicidal individuals by means of interviewing and testing them, and to evaluate, diagnose and refer suicidal individuals for treatment to various resources in the community; to receive back from the community treatment resources and correlate all treatment data with subsequent records of individuals who attempt to commit suicide in the community; and to study the effectiveness of the methods of treatment used in the community.

PSYCHOSOMATIC ILLNESS

Does the specific attitude of a patient toward a disturbing life situation bear any relation to the disease or symptoms he develops? There has been some clinical evidence that this is true, and now NIMH grantees, Drs. David T. Graham, John A. Stern, and George Winokur at Washington University's School of Medicine have developed experimental evidence in support of the "specificity of attitude" hypothesis.

The investigators proceeded on the premise that if a specific attitude is, in fact, associated with a given set of physiological changes, it should be possible to induce certain attitudes experimentally in healthy persons, and to determine whether these attitudes result in the expected physiological changes. The psychological and physiological correlates of two psychosomatic diseases--hives and Raynaud's disease--were chosen as the subject matter for the experiment. Each of these disorders has previously been reported by clinicians to be associated with distinct attitudes. The hives sufferer, when he perceives himself as being mistreated, typically does not develop even a wish to take some action himself, while the person with Raynaud's disease wishes to take some direct, hostile action when mistreated. Physiologically, each of these disorders is associated with a specific, measureable change. Persons with hives undergo an increase in skin temperature, while those suffering from Raynaud's disease experience a cooling of the skin.

Twenty-four young, healthy males volunteered as subjects for the experiment. They were hypnotized, and, using a variety of methods, the investigators attempted to induce the typical "hives" or "Raynaud's" attitudes. Through hypnotic suggestion they encouraged either a "take your mistreatment without fighting back" or a "fight back against mistreatment" frame of mind. Changes in skin temperature were recorded during the hypnotic sessions, and these readings were related to the attitudinal data. The results lend experimental support to the clinically derived hypothesis that physiological changes are specific to the patient's attitudes. In this instance there was a significant difference in skin temperature, in the expected direction, between those subjects who were encouraged to assume the "hives" and "Raynaud's" attitudes.

The broad significance of this study lies in the weight it lends to the view that patients with various psychosomatic diseases can be differentiated in terms of the specific stance they take toward disturbing life situations. The study was reported in Psychosomatic Medicine.

THE GENERAL PRACTITIONER AND MENTAL HEALTH

In September 1958, the first official announcements describing the Institute's psychiatric training program for general practitioners were issued. Within six months the total funds allocated for the program were obligated. Under the program, the Institute is presently providing support for general practitioners who are taking psychiatric residency training, and postgraduate psychiatric training courses for general practitioners.

The importance of these training activities is highlighted by an estimate by Dr. Charles E. Goshen, Director of the American Psychiatric Association's General Practitioner Education Project, which is financed by an NIMH grant, that at least 90% of all psychiatric problems receiving medical attention are handled in the office of the family physician. Dr. Goshen sees the mental health role of the general practitioner (family physician, internist or pediatrician) as being primarily preventive. The first level of prevention is conceived of as preventing the development of emotional problems. At this level the general practitioner would help patients, schools, and the community learn the most effective ways of raising children. The second level of prevention is to recognize danger signals indicating emotional disturbances in children and to attempt corrective action. A third level of prevention occurs during adolescence and early adult life when physicians can help troubled people make decisions at critical periods in their school, career, and married life. The fourth level of prevention lies in helping

people with full-fledged psychiatric problems to avoid additional complications (e.g., drug or alcohol addiction, unnecessary operations, divorce, suicide, etc.). The fifth level of prevention is attempting to rehabilitate people who have already been institutionalized or have succumbed to other forms of helplessness.

The recommendations for a training program made by the General Practitioner Education Project are that: (1) each physician become familiar with the basic principles of psychiatry as he has with other medical specialties; (2) he acquire this familiarity through postgraduate education; (3) medical organizations recognize psychiatric disability as the largest single public health problem today; and (4) medical organizations assume the leadership necessary to meet the need for this type of medical postgraduate education.

PUBLICATIONS

Several important publications, including proceedings of outstanding conferences in the field of mental health, were issued during 1959.

Proceedings of the Conference on Research in Psychotherapy (American Psychological Association, 1959) summarizes the results of a three-day conference held in Washington, D.C., in April 1958, under the auspices of the Division of Clinical Psychology, American Psychological Association, with support from NIMH. The conference provided a forum where some of the leading investigators were able to take stock of the present status of research in psychotherapy. By focusing on specific key topics, the participants were able to make critical evaluations of objectives and methodologies. The conference papers, and discussions which followed, were concerned with both comprehensive descriptions of some of the large-scale research programs as well as extensive analysis of crucial research problems.

The Thematic Apperception Test: An Interpretive Lexicon for Clinician and Investigator (Journal of Clinical Psychology, Monograph Supplement No. 12, April 1959), summarizes interpretive statements that have been made about subjects' responses to the Thematic Apperception Test (TAT), a psychological projective test. The lexicon was compiled by NIMH grantee Gardner Lindzey with the assistance of Jean Bradford (University of Minnesota), Charlotte Tejessy (Massachusetts General Hospital), and Anthony Davids (Brown University). The TAT is an important instrument for the clinician in diagnosing mental disorders, as well as for the research investigator concerned with learning more about human personality (normal as well as pathological).

A significant step forward in the growth of interdisciplinary research in the field of mental health is represented by the publication Biological and Biochemical Bases of Behavior edited by Harry F. Harlow and Clinton N. Woolsey (University of Wisconsin Press, Madison, Wisconsin, 1958). This contains the proceedings of the Symposium on Interdisciplinary Research which was supported by a grant from the NIMH. The papers presented at the Symposium deal with the correlation of behavioral data with data obtained from other biological sciences--anatomy, embryology, physiology, pharmacology, and biochemistry. The papers range from fairly detailed reports of individual research to broad studies dealing with the fundamental problems of biology and behavior. Many of the papers report on interdisciplinary experiments involving a combination of behavioral and other biological techniques. The volume is a good example of the interdisciplinary approach and represents an important contribution to the growing body of studies on brain and behavior. Contributors to the Symposium include outstanding neurophysiologists, psychologists, psychiatrists, and representatives of other mental health disciplines. A number of the studies presented in the Symposium were supported by NIH grants.

A comprehensive three-year study of curriculum in the education of social workers has been completed under the auspices of the Council on Social Work Education with partial support from the National Institute of Mental Health. The curriculum study consisted of twelve separate projects, each of which has been reported in a separate publication issued by the Council. An introductory volume entitled Objectives for the Social Work Curriculum of the Future contains the over-all goals. The NIMH provided support for the volume on Human Growth and Development.

The special role and the invaluable contributions of the volunteer worker in dealing with the problem of mental illness are analyzed in a recent publication of the American Psychiatric Association entitled The Volunteer and the Psychiatric Patient (American Psychiatric Association, Washington, 1959). The volume is a report of the Conference on Volunteer Services to Psychiatric Patients held in Chicago in June 1958 by the American Psychiatric Association, the American Hospital Association, the American National Red Cross, the National Association for Mental Health, and the Veterans Administration, under a grant from the National Institute of Mental Health. The report of the conference provides information about the nearly 43,000 volunteers who work exclusively with psychiatric patients throughout the nation. It tells who they are, where they work, and what they do; discusses some of the administrative and other problems encountered in the utilization of volunteer services; and it includes a set of principles to guide progress in the development and improvement of volunteer programs.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON NEUROLOGICAL AND SENSORY DISORDERS

1959

Items of Interest on Program Developments and Research Studies

Conducted and Supported by the National Institute of Neurological Diseases and Blindness

The research program of the National Institute of Neurological Diseases and Blindness expanded during 1959 and progress relating to major neurological disorders was reported by investigators at the Institute and at medical centers receiving research grants.

The scientific developments presented on the following pages are selections from the Institute's research achievements relating to the brain and central nervous system. They form a mosaic of additions to the field of neurological knowledge -- both laboratory and clinical -- which provide hopeful clues to solutions of single ailments and groups of disorders.

All the accomplishments listed here have appeared as published research papers or have been presented at scientific and professional meetings.

CENTRAL NERVOUS SYSTEM

MECHANISM OF SPREAD OF TETANUS TOXIN STUDIED

The possibility that tetanus toxin may reach the central nervous system via tissue spaces

in the inner layer of connective tissue in the sciatic nerve (perineurium) was revealed by an NINDB grantee study.

The study was conducted to resolve some problems relating to the communications between the periphery and central nervous system by Drs. Alexander A. Fedinec and Howard A. Matzke, University of Kansas, and was reported in the AMA Archives of Neurology and Psychiatry.

Although extensive research has been done on the pathway of the neurotropic agent (tetanus toxin) to the central nervous system, considerable controversy still exists as to whether an element of the peripheral nerve or the vascular system is the primary route.

Experiments were conducted on adult rats some of which were injected intramuscularly and some intravenously with tetanus toxin. Intramuscular injections produced local tetanus and, at a later period, generalized tetanus which was presumably blood-borne since this later could be prevented by intravenous antitoxin. Measures which damaged the perineurium interfered with the appearance of local tetanus.

Degeneration of the nerve fibers did not prevent the appearance of local tetanus in adjacent muscles. However, the spread was delayed. Removal of the outer layer of connective tissue in the sciatic nerve (epineurium) did not prevent the development of local spread. However, partial removal of the perineurium prevented local tetanus and led to systemic tetanus only.

NERVE IMPULSE TRANSMISSION STUDIES MAKE SIGNIFICANT CONTRIBUTION

An NINDB study of nerve impulse transmission confirms the possibility of an additional synaptic relay being involved

in the inhibitory pathway of cells of the central nervous system.

The study was conducted by Dr. Karl Frank, NINDB's Neurophysiology Laboratory and Dr. James M. Sprague, University of Pennsylvania Medical School. Their findings were reported in Experimental Neurology.

The purpose of the experiment was to resolve the important question of whether so-called "direct" inhibition in the central nervous system involves an extra inhibitory interneuron to account for latency of the inhibitory response in nerve cells. The dorsal and ventral roots of the 2nd and 3rd sacral segments of anesthetized cats were exposed and cut bilaterally at equal distances from the cord for stimulation and recording. Search was made in these sacral segments with microelectrodes for motoneurons which responded to stimulation.

The investigators reveal that despite the brief latency observed with "direct" inhibition there was just time for one extra synapse, and, hence, an interneuron. This finding supports the contention of other scientists engaged in similar experiments of nerve impulse transmission, namely, that inhibition is accomplished by interneurons.

This study is a noteworthy contribution toward further understanding of the anatomy and physiology of nerve cells and their mechanisms of interaction.

RESPONSES OF NERVE MEMBRANE IN SQUID GIANT AXON

Further studies at NINDB on ionic membrane current measurements in the squid giant axon

show that the control of the membrane potential may be somewhat more difficult to achieve than previously realized. A lack of this control may be responsible for occasional distortions that have been observed in the current patterns.

The continuing work on ionic membrane currents by Drs. K. S. Cole, J. W. Moore, and R. E. Taylor, NINDB's Laboratory of Biophysics, was reported at the National Academy of Sciences annual meeting. An abstract of this report appeared in Science.

Using earlier techniques for measurement, variations in the current patterns had been observed and a re-investigation was conducted on the conditions under which a nerve membrane can be adequately controlled. It was found that axons usually produced ion current patterns of the type originally described by Dr. Cole and analyzed by Hodgkin and Huxley when the membrane potential was well-controlled and when the axons were in the best condition to give their maximum responses.

In reporting on current research, the effect of the internal electrode characteristics on the observations was emphasized. The surface resistance of the axial electrode was found to be the most critical single factor and the occasional obvious distortions of the current patterns are attributed to the marginal adequacy of the internal electrode in combination with the external electrode.

SYMPATHETIC NERVOUS SYSTEM ACTIVE IN PUPILLARY DILATION IN DARKNESS

As a result of experimentation on the eyes of cats, NINDB investigators conclude that sympathetic nerve fibers do

not merely contribute tonus to the iris, as reported by others, but that they actively contribute to dilation of the pupil in response to decreased illumination.

Drs. Clark J. Bailey and Lloyd Guth, of the Laboratory of Neuro-anatomical Sciences, NINDB, reported their findings in Experimental Neurology.

Although the pupil dilates in darkness and upon stimulation of the cervical sympathetic trunk, it had not been shown, according to the investigators, that darkness causes a reflex action in

the sympathetic nervous system. In the present experiment, measurements of cats' pupils were made from photographs taken in light and darkness. Atropine was instilled into the eye to block parasympathetic action. It was found that the atropinized pupil is capable of a small, but significant, dilation in darkness.

Later, the sympathetic trunks of the cats were transected. The animals were atropinized and photographed one week after surgery. The sympathectomy greatly reduced the dilation under atropine, for a statistically significant difference was noted between the atropinized normal and atropinized sympathectomized pupils.

This difference indicated to the investigators that a significant amount of the dilation of the atropinized normal pupil results from the action of the sympathetic nervous system. Thus, they conclude that the sympathetic fibers are active in maintaining the size of the dark-adapted pupil.

LACK OF BLOOD SUPPLY IN
SPINAL TRAUMA MAY BE THE
CAUSE OF SERIOUS NEURO-
LOGICAL DISABILITY

Some cranio-cerebral deaths
following trauma for which
no cause of death can be
found intracranially may
result from a lack of

vertebral blood supply to the brain stem and cervical spinal cord, Drs. Richard C. Schneider and Elizabeth C. Crosby, NINDB grantees of the University of Michigan and St. Joseph Mercy Hospital, reported in Neurology.

In a meticulous study of the circulation in the spinal cord and its reaction to injury, the authors demonstrated that in many patients, apparently suffering from head injury, the neurological impairment is actually the result of spinal cord damage due to compression and injury of the spinal arteries.

To illustrate the problems in properly diagnosing such cases, the histories and diagnostic problems posed in five such patients were reviewed. All patients suffered severe neurological disability. In three, there was vascular insufficiency of the brain stem; in one, of the cervical cord; and in the other, impairment of the blood supply to the thoracic spinal cord.

After a definite diagnosis of vascular insufficiency is made, prompt application of cervical traction is the best method of relieving pressure on the vertebral arteries, according to the authors. Also an early tracheotomy may be advisable in high cervical dislocation cases, to prevent further neurological disability from hypoxia.

In conclusion, Drs. Schneider and Crosby emphasize the need for more adequate clinicopathologic examination of the cervicomedullary region in such cases.

CELL GROWTH AND MIGRATION TRACED IN EMBRYONIC MOUSE BRAIN

Autoradiography with tritium-labeled thymidine provides a method of tracing cell

development in the relatively inaccessible mammalian embryo, reported Drs. Richard L. Sidman, Irene L. Miale, and Ned Feder, formerly of the Laboratory of Neuroanatomical Sciences, NINDS, in Experimental Neurology.

In the present study, cell proliferations and migrations in the primitive ependymal zone were considered, since differences of opinion have been expressed regarding the nature and behavior of cells in this zone.

Tritium-labeled thymidine (thymidine- H^3) was found to be the most desirable marker because thymidine- H^3 is incorporated into deoxyribonucleic acid (DNA) of cells preparing for division and the tritium remains thereafter in those cells and their progeny.

Pregnant mice were injected intravenously with thymidine- H^3 and killed at various intervals. Autoradiograms were prepared of sections through the embryonic brains. Eleven-day embryos fixed 1 hour after exposure to thymidine- H^3 showed heavy labeling of most cell nuclei in the external half of the primitive ependymal layer in the wall of the cerebral vesicle, and almost no labeling in the inner half. Therefore, according to the investigators, the external half of the primitive ependymal layer is the primary site of early DNA synthesis.

In conclusion, the authors state that the embryonic cerebral vesicle provides excellent material for analysis of the mitotic cycle because it presents a fairly uniform population of cells with a distinct spatial separation of the process of DNA synthesis (in the outer half of the primitive ependymal zone), from the process of cell division (in the inner half). In addition, the cells proceed fairly synchronously through synthesis of DNA, migration, and division.

The conclusions of this report confirm views of investigators conducting similar research with chick embryos.

BRAIN AND CEREBRAL CORTEX

NEW FACTORS IN BRAIN METABOLISM

Two compounds, gamma-aminobutyric acid and succinic semialdehyde, have been conclusively shown to support brain metabolism in vitro. Formerly, it was thought only

glucose or glutamic acid could perform this metabolic function.

Drs. Guy M. McKhann and Donald B. Tower, NINDB Clinical Neurochemistry scientists, noted that the two compounds form a pathway of metabolism in the brain which may be interfered with during certain types of seizure states. Their findings were reported in American Journal of Physiology.

The role of these two compounds, present in the brain only, was demonstrated during studies of slices of cat cerebral cortex to which the two compounds, along with others, were added for the purpose of observing their effect on oxygen consumption.

The authors conclude from the results of their investigation that the role of gamma-aminobutyric acid, present in relatively large amounts in the brain, should be re-examined. The relatively high concentration in the gray matter and active metabolic participation suggests gamma-aminobutyric acid may regulate a portion of the available energy and thus affect levels of functional activity within the brain.

BRAIN ACTIVITY OF MONKEYS AFFECTED BY ELECTROMAGNETIC WAVES

Neurological changes and even death occurred in animals following exposure of the cerebral structure to electro-

magnetic waves of a frequency of 388 megacycles in experiments at NINDB.

The effects of electromagnetic waves on the brain activity of monkeys were studied by Dr. Maitland Baldwin, Dr. Sven A. Bach and S. A. Lewis, R. N., NINDB Surgical Neurology Branch. Their findings were reported at the annual meeting of the American Psychiatric Association, and during hearings before the House Sub-Committee on appropriations. Although similar experiments have been conducted, none have described the effects of frequencies in this specific range, nor the effect of exposure to the head alone.

The experiments proceeded by fastening the animal to a chair in a sitting position within a drum-shaped cage of copper mesh. An insulating collar to prevent burns was placed around the monkey's neck. A radio antenna was fitted to the top of the cage directed toward the monkey's head in line with his brain stem. No direct connection between any current-carrying device and the animal was used. A motion picture camera was placed in front of the monkey to televise his posture and movements. Further behind the camera, a radio was attached to a transmitter. The cage was designed so that exposure could be made while the head was held either high or low within the cavity. Each animal in the experiment was exposed

to continuous waves from the transmitter with an average duration of exposure between two and ten minutes. Electroencephalograms, respiration rate, blood pressure, pulse rate and rectal temperatures were recorded before, during and after exposure. The animals did not receive any medication during this period. Shortly after the transmitter was switched on, the animals became agitated, drowsy and akinetic. Eye changes were then apparent along with an increase in the respiratory rate. Salivation and grimaces followed accompanied by labored breathing. After these changes, a generalized seizure occurred. In most cases, changes in the scalp electrogram were evident approximately 140 seconds after exposure began. Each phase or stage in the sequence was punctuated by a marked change in the monkey's awareness and posture.

The nature and severity of clinical changes varied with the position of the head. It was found possible to kill monkeys with an exposure of only two minutes and 55 seconds when the head was held in the proper position. In addition, when the whole animal except for the head was exposed for ten minutes at 388 Mc. no changes were observed.

Not all monkeys were given maximum treatment as exposure was stopped when changes indicative of eventual death were observed. Those who survived showed clinical signs of varying degrees of brain damage, but 48 hours after exposure clinical signs disappeared.

Gross inspection of the brains of monkeys who did not survive revealed no pathological cause for death, but there was some histological evidence of an intraneuronal disturbance. In some sections there were no apparent abnormalities, but in others there was some "fading" of the Nissl substance. This, according to the investigators is considered to be the result of molecular changes subsequent to the electronic activation by this specific frequency.

The implications of these experiments may prove to be far-reaching. It may be a valuable research tool. It may be used in the experimental stimulation of various brain functions. It might be useful in prevention of brain dysfunction or serve as a basis for important psychological experiments.

NEUROPATHOLOGICAL RESULTS OF ASPHYXIA NEONATORUM IN THE MONKEY

Neuropathological examinations of the brains of five monkeys asphyxiated for varying periods of time showed primary neu-

ronal changes and to a lesser extent, destruction of neuroglia cells.

A study of the effects of asphyxia neonatorum in monkeys was conducted by Drs. William F. Windle, Chief, NINDB's Laboratory of Neuroanatomical Sciences, James B. Ranck, Jr., Department of Physiology, University of Washington, Seattle, and their associates at the Field Station for Perinatal Physiology, Puerto Rico. A report of the study entitled "Brain Damage in the Monkey, Macaca Mulatta, by Asphyxia Neonatorum" appeared in Experimental Neurology.

Asphyxia neonatorum was induced in five monkeys near term by removing the uterine contents intact and waiting until respiratory efforts ceased or were about to cease before freeing the infant monkeys from the fetal membranes. Eleven to sixteen minutes later the fetuses were delivered from their membranes and resuscitated by inflating their lungs with oxygen. Two others were delivered at once to serve as normal controls.

The monkeys were killed from periods of two to nine days by perfusion-fixation which produces instantaneous death of cells in all parts reached by the vasculature. The procedure gave a high degree of uniformity in histologic preparations.

The asphyxiated monkeys displayed definite neurological deficits during life. The lesions resulting from the effects of asphyxiation resembled in distribution the lesions seen in kernicterus.

The histologic preparations of the brains of these primates presented a single neuropathologic entity, differing mainly in gradations of intensity and time. They were similar in respect to symmetry of nuclear distributions of damage, with certain nuclei constantly involved.

The primary effect of asphyxia was cytolysis (dissolution or destruction of cells) and associated injury and loss of neurons and neuroglia. The brains of the two oldest monkeys showed similar cytolysis and loss, but in addition, there were marked reactive changes in astrocytes, microglia cells and walls of the blood vessels.

Acute anoxia may not be the only or even the primary cause of the neurological damage observed, according to the NINDB scientists. The associated excess of carbon dioxide in the blood, cardiovascular alterations, biochemical changes, singly or in combination, may be other precipitating factors.

It is hoped that these studies will form the basis of a better understanding of neuropathologic conditions in human infancy thought to be attributable to asphyxia before or during birth, but for which a clear relationship is lacking at the present time.

ABLATIONS OF SALAMANDER
BRAIN MODIFY DRUG-INDUCED
CONVULSIONS

NINDB grantee investigations added further support to the view that during phylogenetic and ontogenetic development,

the higher centers of the central nervous system do not replace the functions of lower centers, but rather successively superimpose on them a more refined regulatory control.

The study of the modification of Metrazol-induced convulsions by ablations of the salamander brain were conducted by J. J. Peters, Ph.D., A. R. Vonderahe, M. D. and K. N. Hehman, B. S. of Xavier University, Cincinnati and University of Cincinnati. The results are reported in Neurology.

Ablations were performed on adult salamanders to test the influence of five major embryologic divisions of the central nervous system - telencephalon, diencephalon, mesencephalon, rhombencephalon and spinal cord - on induced seizures. Neurological tests done five or ten minutes after ablation were designed to determine the function of various pathways in the nervous system. Metrazol was injected into the abdominal cavity, with a few drops applied to the region of ablation. The reaction of the salamander to Metrazol was compared with that of an intact salamander undergoing a characteristic seizure.

After ablation of the telencephalon, the salamander had coordinated walking, righting and swimming, corneal and abdominal reflexes, and positive responses to tactile and visual stimulation. Intra-abdominal injection of Metrazol induced seizures with tonic and clonic components, hypersensitivity, gasping and writhing. After ablation of the prosencephalon, Metrazol induced a seizure with an extreme tonic phase but hypersensitivity and the clonic phase were lost. When both the prosencephalon and mesencephalon were removed, the salamander could still walk, right itself and swim, but Metrazol failed to evoke seizures with tonic and clonic components. The salamander still had an abdominal reflex and withdrew the limbs when touched after the entire brain was excised, but injection of Metrazol was followed mainly by writhing and erratic placement of limbs.

As ablations proceeded posteriorly eliminating the higher centers, the salamander had successive deficiencies in such refined actions as walking, righting and swimming until only gross writhing of the vertebral column remained. The experimental results of this study seemed to establish the fact that writhing is the basic movement on which a placement of limbs is superimposed. A motor zone in the medulla marshalls these simple movements into acts of swimming, walking and righting. Finally, the mid-brain and forebrain, with centers for sensory correlation, integrate these activities to move and adjust the organism to environmental changes.

The data supplied by studying the salamander may supply clues for a clearer understanding of convulsive phenomena in higher forms.

UNUSUAL NINDB STUDY CONFIRMS
THEORY OF LOCATION OF PER-
CEPTION IN THE BRAIN

Studies with chimpanzees gave further evidence that the lateral temporal cortex of the brain is concerned with

perception. Lysergic acid plus various surgical techniques administered to chimpanzees over a two-year period provided the new information.

The experimental study administering lysergic acid to chimpanzees was made by Drs. Maitland Baldwin and S. A. Bach and S. A. Lewis, R. N. of the Surgical Neurology Branch, NINDB. Their report appeared in Neurology.

During this experiment, 15 healthy young chimpanzees of an average age of 7 years were observed. Before these experiments each animal was observed for at least one year to condition it to handling and hand-feeding as well as to close observation. Surgical operations were performed in different areas of each animal's brain. The methods and circumstances of the drug preparation and administration were similar or identical in all experiments.

The experiments showed that the young chimpanzee reacted to lysergic acid with a panorama of abnormal behavior which was consistent and characteristic. The reactions to the drug presented a compound of perceptual aberration and panic. As the animal reacted, he might look at his hand and scream. The pupils dilated, his hair stood on end and he reacted abnormally to the usual forms, colors and sounds of his habitual surroundings.

An animal subjected to a bifrontal lobectomy displayed a radically changed reaction to ordinary situations but an unchanged reaction to the drug. Of all postoperative responses, only the drug reaction remained comparable to those observed prior to operation.

At the same time, if an animal was subjected to a bilateral temporal lobectomy, his responses to ordinary situations remained remarkably unchanged except for a four-week period after surgery, but his response to the drug was obviously changed.

Unilateral temporal lobectomy did not change the animal's reaction to lysergic acid, nor did such surgery alter social or training responses. According to the NINDB scientists, one or the other temporal lobe is essential for this drug reaction in young chimpanzees.

When the temporal ablation included only the structures situated in the inner margin of the temporal lobe, the reaction to lysergic acid was unchanged. Only after selective ablation of both lateral temporal cortices, did the reaction either change or disappear.

The reactions to lysergic acid both in humans and chimpanzees are characterized by gross perceptual disturbances. Since removal of the lateral temporal cortices prevented gross perceptual aberrations in the chimpanzees, it was suggested that the drug disturbs the perceptual mechanisms present in the gray mantle of the temporal lobe.

CEREBRAL EMBOLISM INDUCED IN ANIMALS THROUGH ADMINIS- TRATION OF RESIN

Methyl polysiloxane resin
(Antifoam A) used in the de-
foamation of oxygenated blood
used for transfusion during

cardiac surgery was found to create cerebral embolisms when injected into dogs.

Drs. J. Kiffin Penry, A. Robert Cordell, Frank R. Johnston, and Martin C. Netsky, NINDB grantees from the Bowman-Gray School of Medicine of Wake Forest College, Winston-Salem, reported their studies in The Journal of Thoracic Surgery.

The advent of open cardiac surgery has revived interest in the study of extracorporeal oxygenation of blood. After bubble oxygenation, however, the blood must be adequately defoamed before transfusion. The usual method used is the passing of blood over surfaces coated with Antifoam A.

In some cases of cardiac surgery employing this method, cerebral complications have been observed. Many investigators, according to the authors, have suspected some of these complications result from traces of Antifoam A remaining in the defoamed blood. During the present study, the cerebral effects of Antifoam A were observed when injected directly into the internal carotid of the dog.

Antifoam is an oily substance with many physical properties similar to those of animal fats and oils, but it differs chemically. Sudden death, with occlusion of the small blood vessels, has been observed after intravascular injection of oil. It is not surprising, the investigators state, to find embolic occlusion of blood vessels after intravascular injection of antifoam in this experiment. The primary lesion in antifoam embolism was the occlusion of capillaries and small arterioles. Immediate death, or coma and subsequent death of the animal result if many adjacent capillaries are occluded suddenly in a large part of the cerebral cortex.

Abnormal clinical signs and histologic lesions were found in all animals receiving an injection of antifoam, but were not seen in the experimental control animals. The macroscopic findings ranged from simple vascular dilatation to frank infarction. The findings on microscopic examination ranged from neuronal changes to infarcts with hemorrhage. Scattered foci of neuronal loss were seen in the cerebral cortex. Small zones of demyelination were present less frequently in the subcortex. Both gross and microscopic lesions were concentrated in the cortex of the parieto-occipital regions and were sparse in the frontotemporal regions. Lesions were rare in the basal ganglions. Antifoam emboli were distributed widely throughout the cerebral cortex of the involved zones and were found infrequently in the subcortical white matter.

The investigators concluded their report by pointing out that the clinical and pathologic findings in cerebral embolism with antifoam are comparable in many respects to those observed in cerebral fat embolism.

CEREBRAL CHANGES CAUSED BY
INDUCED FAT EMBOLI IN
ANIMALS COMPARED WITH
SIMILAR CHANGES IN HUMAN
SPECIMENS

Fat emboli, induced experimentally in animals, have been found to cause varying degrees of paralysis and decerebrate rigidity. As in human specimens, the

cerebral changes varied considerably in appearance.

In the study, conducted by Dr. Jan Cammermeyer, Laboratory of Neuroanatomical Sciences, NINDB, and Dr. Roy L. Swank, Neurology Division, University of Oregon Medical School, eight adult dogs were given intravenous injections of Wesson oil and later sacrificed at intervals from 22 hours to 9 days. A detailed report of the investigators' findings appeared in Experimental Neurology.

The symptoms following the induced fat emboli ranged from a temporary or even permanent decerebration to varying degrees of paralysis and ataxia. Two animals showed no neurological deficit. The absence of symptoms, however, did not preclude tissue damage, but histological changes were difficult to correlate with clinical signs. There was no direct relationship between the severity of symptoms and changes in the vascular system. Clinical symptomatology was found to be severe in three animals with pronounced anatomical changes.

Examination of the specimens showed Sudan-stained fat emboli scattered through the brain, accumulated within circumscribed areas and around foci of degeneration. Lesions of the brain differed in size and degree of tissue destruction. An excessive deposit of iron in the tissues occurred from the second day and was more evident in the cerebellum than the cerebrum. Fibrin was found around the vessels near the embolized part in two-day old lesions. The glomeruli were clogged by separate threads of fibrin attached to fat emboli. After repeated injections of fat, the cerebrum and cerebellum were damaged by hemorrhagic infarctions. Otherwise all foci seemed to have developed around vessels with arrested emboli. The factors or mechanisms involved in these changes are not understood.

The experiment also was an attempt to re-evaluate the significance of certain tissue changes in human specimens based on the information obtained from this particular study.

SYNCHRONIZATION OF
CEREBRAL CORTEX CELLS
STUDIED

Experiments at NINDB substantiated with limiting considerations a previous notion that groups of nerve

cells in the cerebral cortex often tend to act in unison when there is nothing to prevent them from doing so.

These studies were conducted by Dr. Choh-Luh Li, NINDB's Surgical Neurology Branch. His findings were reported in Science.

The activity of cortical neurons was simultaneously recorded with two micropipette electrodes from the somatosensory cortex of cats. The tips of the microelectrodes were estimated to be less than 0.5 mm apart. When a microelectrode picked up spike activity, it was left undisturbed while the other microelectrode was used to study similar activity of other nerve cells within a sphere approximately 1 mm in diameter. Toward the end of each experiment strychnine solution was applied as a stimulant to the area of the cortex where the electrodes were inserted.

"Spontaneous" discharges of the cortical units recorded from cats with transections of the midbrain and from cats under deep general anesthesia were remarkably similar. Recordings from cats under light general anesthesia showed many units discharging also in bursts, but the time relationship between the bursts from different units was less clear. Although synchronous discharges of the two units were not observed, there was a tendency for the discharges of the units to pause at about the same time and for the periods to be of similar length.

Stimulation of the peripheral nerves elicited responses of variable latencies. Application of strychnine caused, in most instances, repetitive discharges in nearly synchronous action.

From these observations it was concluded that units within a sphere of 1 mm diameter in the cerebral cortex seldom fired at precisely the same instant. When the cortex is aroused the unit activity was previously said to be desynchronized, but in this study a relationship between the discharges of the units still existed.

SIGNIFICANT ROLE OF CHOROID
PLEXUS IN FORMATION OF
CEREBROSPINAL FLUID
CONFIRMED

New findings relating to the
cellular, chemical, and
enzymic activity of the tissue
of the choroid plexus and its
role in the formation of

cerebrospinal fluid may lead to advances in the management of intracranial fluid problems such as hydrocephalus.

Drs. Robert G. Fisher and John M. Copenhaver, Jr., NINDB grantees, Dartmouth Medical School, found the histological structure of the choroid plexus well designed for the active or passive transfer of constituents of blood. Their extensive study using cats produced definitive evidence that choroid plexus tissue can produce or absorb cerebrospinal fluid. The results of this study appeared in the Journal of Neurosurgery.

Histochemically, the ependymal (epithelial) cells of the plexus were found to be rich in succinic dehydrogenase and carbonic anhydrase while the blood vessels had a high concentration of alkaline phosphatase. All three of these are believed to play active roles in the enzymic activity of this tissue.

The biochemical analysis indicated that the general metabolic activity is one-third to one-half of that of the kidney.

The effect of Diamox, a carbonic anhydrase inhibitor, was also studied for its effect on that enzyme in the choroid plexus and the blood. Diamox reduced the flow of cerebrospinal fluid regardless of the dose of the drug used. Direct correlation existed between the dosage of the drug and carbonic anhydrase activity of the blood. The flow of cerebrospinal fluid, however, was not directly correlated with inhibition of carbonic anhydrase of the choroid plexus.

INFANT ABNORMALITIES

BRAIN LESION PATTERNS CORRELATED WITH PATIENTS' HISTORIES

Two different types of clinical histories, perinatal and post-natal, were found to correlate respectively with two contrasting pathoanatomical patterns of lesions in 162 cases of brain damage in children.

The cases selected for study by Dr. N. Malamud, NINDB grantee, University of California at San Francisco, appeared first to be etiologically and pathoanatomically non-specific. Further analysis distinguished two fundamentally different patterns of lesions, namely, primary subcortical pathology and primary cortical pathology. The results of these findings appeared in the Journal of Neuropathology and Experimental Neurology.

Among 101 cases of primary subcortical pathology, 86 percent showed a definite or probable history of birth trauma caused by such conditions as difficult labor, placental conditions such as abruptio and placenta previa, asphyxia neonatorum and prematurity. Among 36 cases of primary cortical pathology 90 percent had a history of normal birth and early development followed by an acute illness occurring at some time during infancy or early childhood. Rarely was a history of birth trauma displayed in these latter cases. In a small group of patients with mixed cortical and subcortical pathology the histories were more complicated and suggested the combined operation of several etiologic factors. It is the pattern and localization of the lesion rather than its morphologic appearance which was significant.

Dr. Malamud suggested that the distribution of the lesions in the cases related to perinatal trauma indicated that they were the result of circulatory changes in the area of distribution of the great vein of Galen.

EARLY DEVELOPMENT OF THE HUMAN BRAIN STUDIED

The central nervous system is more commonly affected than any other part of the body in

babies born with congenital malformations.

A detailed study of the early development of the brain was conducted by Dr. Anatole S. Dekaban, Section on Developmental Neurology, NINDB, and Dr. George Bartelmez of the Carnegie Institution of Washington. The results of their work were presented at a meeting of the American Association of Anatomists.

According to the current knowledge, disturbances of differentiation of cerebral structures occur most frequently during the first two months of gestation. The mechanism of the formation of congenital abnormalities can be understood only by reference to the relation and sequence of events in normal development. Therefore, a knowledge of the normal development of the human brain during early embryonic life is a necessary prerequisite for the understanding of numerous, complex malformations of the central nervous system.

Congenital malformations which affect the central nervous system are responsible for a proportion of the patients with cerebral palsy, epilepsy and mental deficiency. Among a few known causes of irregularities of cerebral development are maternal infection with German measles and excessive ionizing radiation during the first ten weeks of pregnancy.

The investigators established and demonstrated detailed landmarks of the longitudinal development of the human brain during early embryonic life. Developmental changes were traced from stages resembling the adult structures to stages in which the entire brain consists of a thin sheath of cells in the form of an open trough. The youngest embryos studied were of only three weeks gestation.

PERINATAL INFECTIONS OF THE CENTRAL NERVOUS SYSTEM DESCRIBED

Considerable damage to the central nervous system may result from infection in

the perinatal period by spirochaetes, protozoa, viruses, bacteria, and other infective agents. Many of these infections are pre-natal and most are comparatively rare.

In a report, Drs. Abner Wolf and David Cowen, NINDB grantees at Columbia University, discussed the variety of infective agents which may enter and damage the central nervous system in the perinatal period. They also assessed the frequency with which these are causes of pathological changes in the brain at this time of life. The report appears in the Journal of Neuropathology and Experimental Neurology.

Perinatal syphilis of the central nervous system is a congenital infection resulting from placental infection and transplacental transmission, probably between the fourth and seventh months of pregnancy. The findings of congenital syphilis at birth are almost never neurological. It is less common for neurological signs of congenital syphilis to appear soon after birth than in the early or late juvenile periods. Congenital syphilis appears to be a diminishing problem as an infection of the neonatal nervous system due to the increasing efficacy of the treatment of this infection.

Congenital toxoplasmosis is a relatively uncommon cause of damage to the brain in the neonatal and infantile periods. Infants suffering from the congenital form of the infection may be stillborn, may have manifest signs at birth or soon thereafter, or seem to be normal at birth and begin to show evidence of the disease in weeks, months or possibly years postnatally. Prominent among these signs are hydrocephalus, chorioretinitis, intracerebral calcification and convulsive seizures.

Acute encephalitis of herpes simplex type in the perinatal period is an uncommon infection of the young, and particularly of the premature infant. The lesions may be disseminated and associated with a viremia or confined to the central nervous system. Polio-myelitis in the neonatal period is very rare. Coxsackie virus infections in the neonatal period are more frequently encountered.

Tuberculosis is sometimes seen in the neonatal period, but neonatal bacterial meningitis is the most common of the perinatal infections of the central nervous system.

Drs. Wolf and Cowen concluded that despite the great number of infective agents which may enter and damage the central nervous system in the perinatal period, infection must still be considered to be one of the less common causes of pathological changes in the brain at this time of life.

PREMATURE BIRTH SURVIVORS STUDIED IN FOLLOW-UP EXAMINATIONS

Premature infants, who received prophylactic antibacterial regimens in the first five days of life, were examined in a follow-up study at two years of age

and a high percentage with brain damage was found. These children had been given either penicillin/sulfisoxazole or oxytetracycline in infancy and at that time an association between a high mortality rate in the premature infants and penicillin/sulfisoxazole was discovered. It is significant that in the study no appreciable difference was evident in the proportion of brain damage between these two groups at the age of two years.

The report of the findings of the follow-up examinations by Dr. William A. Silverman, NINDB grantee at Babies Hospital and Department of Pediatrics, Columbia University, appeared in The Journal of Pediatrics.

The disturbingly high proportion of these survivors of premature birth who exhibited signs of neurological deficit and mental retardation at two years suggested, according to Dr. Silverman, that the problem of brain damage among premature infants is one which requires equal consideration with the problem of reducing the mortality rate in the newborn.

There were 192 premature infants in the original study and 104 infants survived. Only 66 of this number were available for the follow-up examinations.

Various examinations were given but only in the neurological, psychometric, language, speech and dental examinations were abnormal findings revealed often enough to warrant analysis.

Of 44 children in the oxytetracycline group, seven were judged to have neurologic deficit and three had suspicious evidence of such deficit. Of 17 survivors of the penicillin/sulfisoxazole group, two had signs of neurologic deficit, and two manifested signs that were interpreted as suspicious of some neurological defect.

Psychometric examination revealed 14 out of 39 individuals in the oxytetracycline group as being either in the borderline or defective class. There were three such individuals out of 18 in the other group.

Language and speech revealed essentially the same proportion in each group of children who were judged to have retarded speech. A slightly higher proportion of the oxytetracycline survivors had defective tooth development.

RARE CASE OF UNILATERAL BRAIN MALFORMATION REPORTED

Unilaterally undeveloped
brain convolutions (agyria)
were discovered at post-
mortem examination in a

10-year-old cerebral palsied child suffering from congenital hemiplegia. Malformation of the brain is a relatively frequent finding in children with cerebral palsy, but the majority of cases reported have had bilateral brain involvement.

The case history of a patient studied by Dr. E. Clarence Rice, D. C. Children's Hospital and Dr. Anatole Dekaban, NINDB's Surgical Neurology Branch, was reported in the AMA Archives of Pathology.

History elicited from the parents revealed that the mother had an apparently normal pregnancy and delivery although ensuing study suggested that there may have been a birth injury. From the age of nine weeks, the child began to have frequent cerebral seizures which were characterized by stiffening of all extremities and an upward deviation of the eyes. With medication, the attacks decreased in number. Neurological examination at three months disclosed right hemiplegia and left esotropia. At the age of

seven months, when being treated for chronic constipation, she was found to have an abnormally large colon. At the age of 18 months, she began to walk, although with a right limp, and at 2 years began to talk. Between the ages of 3 and 7 years she was seen and treated in the cerebral palsy clinic for right hemiparesis and epilepsy. At the age of 7, during hospitalization for increased difficulty in bowel movements and a poor general condition, a high white cell count revealed chronic myelocytic leukemia. The patient died at ten years of age following uncontrollable convulsions. Post-mortem examination disclosed the following findings: Undeveloped convolutions (agyria) of the anterior part of the left cerebral hemisphere; absence of ganglion cells in the myenteric ganglion, associated with enlarged colon; recent hemorrhagic infarction of the anterior half of the right cerebral hemisphere, and myeloid leukemia.

Agyria is one of the most primitive malformations of the cerebral cortex and is generally considered to be a result of arrested development of the brain before the fetus has reached 14 weeks of gestation. The estimation of the earliest possible teratogenic period is difficult, although it is unlikely that a noxious factor acting as early as the first weeks of prenatal life could lead to this anomaly.

The authors stated that it is of interest that the right hemiparesis and also the epileptic attacks were attributed to a suspected birth trauma, but the possibility of malformation of the brain was not considered at all. Children suffering from congenital malformations have a slightly higher risk of developing leukemia than their normal mates.

KERNICTERUS

ANIMAL STUDIES EXTEND

KNOWLEDGE OF KERNICTERUS

A study of congenital jaundice of rats (Gunn's strain)

revealed similarity to human

kernicterus and extends knowledge of bilirubin formation, intoxication, and excretion.

The report of an extensive study of kernicterus by Drs. William A. Blanc and Lois Johnson, Columbia University and the State University of New York, NINDB grantees, appeared in the Journal of Neuropathology and Experimental Neurology. These investigators found the clinical and pathological symptoms of kernicterus observed in a certain strain of rats were similar to those appearing in human kernicterus. These rats have a congenital deficiency of the enzyme required to convert bilirubin so that it can be excreted by the liver (hypoglucuronyltransferasia).

Pathologically, the most extensive damage was observed in animals which died during the third week. In these, cellular loss was apparent and numerous ganglion cells were destroyed or shrunken. Autopsy of the subjects revealed jaundice in the thoracic and abdominal cavities and in the subcutaneous tissues and serous membranes. All organs were slightly jaundiced and otherwise normal in size and general appearance with the exception of the lungs, kidneys, and intestine.

The investigators studied the effects of sulfonamide administration to these animals. Ordinarily, kernicterus develops after the 7th day of life. However, either with severe infection or with the administration of certain sulfonamides gross kernicterus was observed as early as the 4th day of life. It was clearly demonstrated that the administration of sulfonamides hastens the onset of kernicterus. The sulfonamides had no effect on the non-jaundiced animals. They did not influence the blood-brain-barrier as tested by trypan blue in the normal or jaundiced animal. They observed, however, that sulfonamides caused bilirubin to leave the blood and enter the tissues.

The close parallelism between symptoms and bilirubin in the tissues supports the belief that bilirubin is directly toxic.

The authors concluded that the deleterious effect of sulfonamides in this strain of rats and in premature infants may be explained by the displacement of bilirubin from the blood compartment into the extracellular fluid. It was suggested that a similar mechanism may be operative in kernicterus occurring under other circumstances.

NEUROMUSCULAR DISORDERS

SUBSTANCES IN MUSCLE
DIFFERENTIATED FOR STUDY

Scientists at NINDB have
traced the development of
muscle fibers in live tissue

by the use of fluorescent immuno-chemical staining techniques.

Dr. Igor Klatzo, NINDB, and his associates conducted studies on the distribution and behavior of a protein substance, myosin, in the muscle. Myosin is responsible, in large part, for the relaxation and contraction of muscle. Their findings were reported at a meeting of the Association for Research in Nervous and Mental Diseases.

The observations on the distribution and behavior of myosin in human muscular diseases were derived from muscle biopsies obtained from the clinical service. These observations were supplemented by a study of experimental muscle lesions in rabbits and the localization of myosin in embryonic chick skeletal muscle grown in tissue culture.

The investigators, in addition to being able to study muscle fiber development, found that myosin is persistent even in diseased muscle and is present in phagocytes which ingest the cellular debris. These specific immuno-chemical staining techniques which differentiate one substance from another are becoming increasingly important in research.

MUSCLE BIOPSY BENEFICIAL IN DIAGNOSIS AND PROGNOSIS

Muscle biopsy is imperative
in the understanding and
ultimate prognosis of the

hypotonic infant.

Drs. J. Godwin Greenfield, Tillye Cornman and G. Milton Shy, NINDB, studied 23 cases of congenital or early infantile muscular hypotonia by muscle biopsy and correlated the pathologic findings with the electromyographic and clinical findings. The report of their study appeared in Brain under the title of "The Prognostic Value of the Muscle Biopsy in the 'Floppy Infant'".

Included in the report of their findings was a description of five different pathologic conditions of muscle which can produce the clinical syndromes of weakness and hypotonia at birth or in the early weeks of life. From this study the prognosis could be divided into three groups--progressive deterioration, improvement, and stationary. Thus, infantile muscular atrophy and congenital dystrophy were considered progressive; benign congenital hypotonia showed improvement; and central core disease remained stationary.

Study of muscle biopsies proved to be an effective means of differentiating the types of muscle disorders studied. With this technique, the authors believe a more exact diagnosis and earlier prognosis may be made. In the case of some disorders, for instance, treatment may prove beneficial if started early in the development of the disorder. In the patients studied, the electromyogram findings were of little value in the eventual differentiation and diagnosis.

NEUROMUSCULAR BLOCKING
COMPOUNDS STUDIED FOR
EFFECT ON MUSCLE TENSION

Compounds which block neuromuscular transmission have been found to decrease the contractile response of the muscle when

directly stimulated by electricity.

The experiments of Drs. Richard L. Irwin and Jay B. Wells, NINDB's Section on Clinical Applied Pharmacology, further clarify muscle reactions when compounds are administered. Their findings reaffirm previous scientific investigations which record decreased contractile response. The results of their experiments appeared in the Journal of Pharmacology and Experimental Therapeutics.

In anesthetized rats, a muscle of the leg (gastrocnemius) was dissected from other soft tissue so that a tension response could be obtained without interference from other muscles. Muscle relaxants such as succinylcholine, decamethonium, d-tubocurarine or gallamine were used principally in the experiments. These compounds were administered into a vein separately or concurrently. When neuromuscular transmission failed, the muscles were stimulated alternately every ten seconds through the nerve and through electrodes imbedded in the muscle. In experiments to record reactions of denervated muscle, tensions were recorded from an innervated and denervated muscle of the same rat.

The twitch tension developed by the gastrocnemius muscle when stimulated directly with extremely high voltage was reduced 50 percent following doses of succinylcholine or decamethonium which stopped neuromuscular transmission for thirty minutes. In contrast to the rapid failure of neuromuscular transmission which occurred in two minutes or less with the doses used, the partial block to direct stimulation developed gradually during the first twenty minutes following administration of the drug.

In contrast, tension remained at near control levels when neuromuscular transmission was stopped for a similar length of time by d-tubocurarine or gallamine.

Doses of succinylcholine or decamethonium did not affect the contraction of directly stimulated muscle in which transmission first had been destroyed by d-tubocurarine or gallamine. This protective effect was less pronounced in denervated than in innervated muscle.

In denervated muscle, the partial block to direct stimulation developed more rapidly, progressed to a greater degree and lasted longer than in innervated muscle.

Stimulus response curves were significantly different after the administration of succinylcholine or decamethonium from those recorded after d-tubocurarine or gallamine. At the point of maximum activity of these two compounds, the rate of development of twitch tension and relaxation was the same as in untreated muscle in which tension was reduced a comparable amount by a decrease in stimulus voltage.

ENZYME INHIBITION STUDIED IN RELATION TO NEUROMUSCULAR BLOCKAGE

Experiments on animals at NINDB extended previous findings on the relationship between neuromuscular blockage and the inhibition of enzymes in muscle and plasma. Greater knowledge concerning this chemical relationship would prove helpful in finding more effective pharmacological preparations for the treatment of myasthenia gravis.

Drs. Richard L. Irwin, Jay B. Wells and Henry J. Smith, of NINDB's Clinical Applied Pharmacology Section, designed experiments to provide further evidence on neuromuscular blockage and cholinesterase inhibition. The results of their investigations were reported at the Second International Symposium on Myasthenia Gravis.

Drs. Richard L. Irwin, Jay B. Wells and Henry J. Smith, of NINDB's Clinical Applied Pharmacology Section, designed experiments to provide further evidence on neuromuscular blockage and cholinesterase inhibition. The results of their investigations were reported at the Second International Symposium on Myasthenia Gravis.

Muscle weakness in myasthenia gravis is known to decrease when substances are administered which inhibit the action of certain enzymes. This weakness may involve a defect in the passing of a biological signal from nerve to muscle similar to that produced by neuromuscular blocking agents. In recent experiments, the effects exerted on neuromuscular blockage by inhibiting both muscle and plasma cholinesterase were investigated using irreversible inhibitors of cholinesterase and three neuromuscular blocking agents, each with different pharmacological properties.

When single pulse stimulation of the motor nerve was used, the inhibition of muscle esterase gave an apparent reduction in the blocking action of succinylcholine, a compound which depolarizes muscle membranes.

With the use of multiple pulse stimulation which produced a brief tetanic response in the muscle, the transmission block produced by succinylcholine was prolonged in the muscle with low esterase activity. Similar changes occurred when transmission was impaired

by decamethonium, a compound which is not metabolized by cholinesterase. These findings suggest that the prolongation of a succinylcholine induced transmission block by inhibition of muscle cholinesterase is not primarily related to degradation of succinylcholine by muscle cholinesterase.

When plasma and muscle cholinesterase were inhibited to a marked degree, the succinylcholine block was greatly prolonged. However, under these conditions, the contribution of muscle cholinesterase inhibition to the total block was considered a minor one. The inhibition of plasma cholinesterase had no effect in the blocking activity of decamethonium or d-tubocurarine. The inhibition of muscle cholinesterase totally prevents the blocking of the activity of an amount of d-tubocurarine, which completely abolishes transmission to a muscle when cholinesterase is not inhibited.

ATROPHY PROGRESSES RAPIDLY IN DYSTROPHIC MUSCLE FOLLOWING DENERVATION

Simple atrophy in a dystrophic muscle is shown to be accelerated after denervation, Drs. Betty Q. Banker and D. Denny-Brown,

Harvard Medical School, reported in the Journal of Neuropathology and Experimental Neurology.

The NINDB grant-aided investigators conducted their study of the chemical and histological changes in dystrophic muscle using a unique strain of mice with a congenital degenerative muscle disease. The disorder closely resembles human progressive muscular dystrophy with minor histological differences.

In both dystrophic and normal mice, the sciatic nerve was transected at the point where it enters the thigh, and beyond this point a segment one centimeter in length was removed. After varying intervals of time, the mice were sacrificed and the denervated muscle compared with the same muscle of the opposite limb.

The turnover of creatine, an important organic compound in contraction of muscle, was increased in the dystrophic and denervated muscles as compared with these values in normal control animals. This was measured using C^{14} creatine. The creatine index (grams of creatine per gram of non-collagenous nitrogen) of this compound was lowest in dystrophic denervated muscles, next in dystrophic animals and least in denervated animals.

Histologically, denervation of normal muscle leads to progressive atrophy of the muscle fibers without degeneration. In contrast the dystrophic muscle contains many degenerating fibers, as evidenced

by loss of striation, hyalinization, and infiltration with macrophages. There is also very prominent variation of fiber size in the dystrophic muscle. Denervation of the dystrophic muscle leads to very rapid atrophy of the muscle fibers, and results in more uniform size and smaller fibers than are seen in the other conditions. However, the number of degenerating fibers is not increased.

The experiments suggested that the dystrophic process continues in the denervated dystrophic muscle, and leads to a degree of atrophy not seen in either the normal denervated or the innervated dystrophic state.

MULTIPLE SCLEROSIS

RISE IN BODY TEMPERATURE
CAUSES NEUROLOGICAL CHANGES
IN MULTIPLE SCLEROSIS PATIENTS

Additional neurological signs
appeared in MS patients during
induced hyperthermia and dis-
appeared when the patient was

cooled. The body appeared to adapt to the heating, however, since the neurological signs disappeared before the body was cooled to the temperature at which the changes were first observed.

In a study of 14 MS patients by Drs. Dewey A. Nelson and Fletcher McDowell, NINDB grantees at Cornell University Medical College, New York City, eight gave a history of severe weakness when exposed to radiant heat or hot baths. A detailed report of the investigators' findings have appeared in the Journal of Neurology, Neurosurgery and Psychiatry.

During the course of the study, the patients' body temperatures were raised between 0.5° and 2.5° F. in two different ways--by infra-red lamps and hot baths. The neurological changes most frequently found were related to eye movement and decreased visual acuity. Among the changes observed were nystagmus, diplopia, dysarthria, and upward gaze palsy.

Neurological signs not evident immediately before heat exposure occurred in 13 of the 14 patients: ten showed multiple changes and three showed single signs. The neurological signs which appeared had, with one exception, occurred at some time in the past during an active phase of the illness. The study also indicated that new signs of neurological dysfunction are more likely to occur after elevation of the body temperature when the disease process is active than when in remission.

In an earlier study, the investigators found that 55 percent of patients with diseases of the nervous system other than MS also developed neurological changes with induced hyperthermia. These were usually single signs and tended to occur at higher elevations of body temperature than in patients with MS.

AMYOTROPHIC LATERAL SCLEROSIS

AMYOTROPHIC LATERAL SCLEROSIS PATIENTS ON GUAM FAIL TO RESPOND TO EXPERIMENTAL THERAPY

Vitamin B₁₂ therapy has proved unsuccessful in the treatment of amyotrophic lateral sclerosis patients on Guam. The investigation, however, produced

information which may prove valuable in future research relating to this fatal muscular disorder. The same medication given previously to a small number of ALS patients in the United States had shown some encouraging results.

A report of the experiments of Dr. Samuel J. L. Pieper, former Surgeon of NINDB's Research Unit at Guam, now of the Department of Neurology, Baylor University College of Medicine, Houston, and Dr. William S. Fields of Baylor, appeared in Neurology.

The medication given consisted of four intrathecal injections per month containing 1000 mg. of vitamin B₁₂ and 50 mg. of water-soluble hydrocortisone. Experimental therapy was started in 26 patients, and all but five received a course of at least 16 injections. In spite of medication, 12 patients continued to follow the usual debilitating course of this disease and 6 patients died while 2 patients remained unchanged. The latter cases previously had been diagnosed as progressive muscular atrophy and lateral sclerosis. One person improved after 9 to 10 months, but 3 months later his disease became as severe as at the initiation of the therapy.

The experiment was conducted on Guam because of the high incidence of the disease on the island and the existence of an NINDB research unit there. Experimental therapy for this disease is difficult to undertake because the disease is rather uncommon in the United States and any one physician would have little opportunity to examine a large group of patients in a short period of time.

A simple grading system was devised to select study patients and to determine the patients' progress. The patients were divided into categories according to the severity of their affliction. Highly specific tests were used to evaluate the progression of muscle weakness on a purely objective basis.

There were no acute complications requiring hospital observation during the administration of 517 intrathecal injections of the drug. From autopsies performed on 4 of the 6 patients who died, there was observed no evidence of inflammation in the subarachnoid space and the intrathecal medication was felt to have been harmless. Dense extradural adhesions were found which were believed to be due to the reaction to bleeding from introduction of the needle rather than any inflammatory reaction to the drugs. These extradural changes were considered to have no clinical significance.

Clinicians who have worked on Guam and neuropathologists who have studied autopsy specimens from Guam agree that ALS in that region, is identical with forms of the disease observed in the United States and elsewhere. Therefore, the systems devised for evaluating the progression of the disease and the data obtained on the mode of drug administration provide certain clues which may prove helpful in future research on this disease in other parts of the world.

DISEASE MAY LINK ALS TO INHERITED NEUROLOGICAL DISORDERS

An inherited neurological disorder clinically indistinguishable from amyotrophic lateral sclerosis has been observed in

several generations of two families. Pathologically, similarities between the cases and disorders such as hereditary ataxias indicate that this disease may represent part of a continuum linking ALS to these inherited neurological conditions.

The disease was described by Drs. W. King Engel and Igor Klatzo of the NINDB Surgical Neurology Branch and Dr. Leonard T. Kurland of the NINDB Epidemiology Branch in Brain.

In the first family studied, at least 11 persons representing four generations were affected; in the second, three persons in two generations were afflicted. The genetic pattern of both families indicates that the disorder is probably a dominantly inherited metabolic failure, affecting the neurone cell body and its processes.

The disorder was clinically similar to ALS in that both conditions usually begin in adult life, terminate fatally within one to four years, and show both upper and lower motor neurone involvement. In addition, the majority of neuropathologic findings were compatible with ALS.

However, evidence of features not occurring in ALS, but present in other conditions indicates that these cases may be variations caused by modifying genetic factors. In addition to genetic patterns which are usually absent in ALS, pathological examination of three cases revealed an unexpected pattern of posterior spinal cord demyelination, especially in the middle root zones. The middle root zones are also selectively involved in other inherited neurological conditions such as subacute combined degeneration, a deficiency disease, and the hereditary ataxias, or idiopathic spinocerebellar degenerations. These diseases may have, at the biochemical level, a similar pathogenesis.

On the basis of this evidence, the authors conclude that the cases studied probably represent a variant of ALS. However, further study of the underlying abnormalities of ALS and its variations are necessary to determine if the disorder is a true example of an intermediate form in the continuum of these diseases.

EPILEPSY

STUDIES MADE ON AUTOMATISM
DURING MINOR EPILEPTIC
SEIZURES

Autonomic changes observed
during minor seizures of
patients believed to have
temporal lesions may prove

an aid in determining the location of epileptic lesions.

The thirteen patients studied by Dr. J. M. Van Buren, NINDB Surgical Neurology, had not responded to medical treatment and were under consideration for surgery of epileptic lesions. Medical treatment was withdrawn during the period of study which ranged from one to eight months.

Graphic recordings of the changing autonomic functions during the patient's attacks formed the data for study. From this material, it was possible to discern some general patterns of autonomic action and some evidence regarding the interdependence of autonomic functions under the conditions of the seizure state.

If a change in autonomic functions appeared, the direction of its change in general, appeared rather stereotyped. These changes might be a rise in blood pressure, increase in pulse rate, fall in skin resistance, a change in swallowing, occurrence of ratching, inhibition of gastric motility and impairment of respiratory function.

In charting all the autonomic features of these attacks by their sequence of appearance, a general time sequence was drawn up. A fall of skin resistance and changes in swallowing tended to appear as the earliest features. Respiratory changes were the most common and were evident early, although this change could occur at any point in the attack. Increase in pulse rate, with or without a rise in blood pressure, usually became apparent somewhat later. Coincident with the cardiovascular changes, or even later, came the patient's aura or unconsciousness. In only one case did unresponsiveness precede any autonomic change.

Despite these generalities of time sequence, the temporal pattern of autonomic and clinical change of an attack varied in each individual. The investigator states that this suggests there is a gradual spread of seizure discharge through spatially separated areas where the autonomic signs appear. The report of his findings appeared in Brain.

EEG PATTERNS STUDIED DURING EPILEPTIFORM PAROXYSMS

Electroencephalographic epileptiform discharges (EEG spikes) are now believed to be the expression of a neuronal hypersynchrony.

Although these electrical events have been previously considered to be the expression of such activity, there has been no crucial evidence to support this assumption.

Dr. C. Ajmone-Marsan, Chief of NINDB Electroencephalography Branch, and the late Dr. T. F. Enomoto, investigated this problem extensively and their reported findings appeared in the Journal of EEG and Neurophysiology.

Epileptic foci were produced experimentally on the cerebral cortex of cats by means of local applications of different convulsant drugs. The development of paroxysmal discharges was monitored with routine surface electrodes. Upon the appearance of these discharges, a systematic survey of the behavior of the various units within the different layers of the nearby cortex was carried out by means of Tungsten microelectrodes. A similar method was applied in a few experiments in which paroxysmal discharges were elicited following intravenous administration of different drugs. These surveys were started a few minutes before the application of the drug, or before its injection, in order to evaluate the "spontaneous" activity of the various elements. Particular emphasis was placed on the behavior of the unit after the epileptogenic effects appeared in the gross surface electrodes.

Although the paroxysmal discharges were, in general, very characteristic and easily recognizable, a definite variability was always present in their morphological pattern, as well as in their amplitude, duration, and polarity. This was true whether induced by the same drug and recorded at different intervals after the onset of the effect or whether they were induced by different drugs. By continuous recording, it was possible to study the behavior of a large number of units firing in coincidence with a relatively stable and constant type of surface discharge, and to compare the behavior of the units in the same or different placements for several types of EEG discharges.

There seems to be almost general agreement, according to the investigators, on the two most common characteristics of the epileptic activation of single units; namely, increase in frequency firing generally in bursts of variable duration and a tendency toward synchronization of the activity of a large number of units. The present study further confirms the previous findings of other investigators. Although the hypersynchronization found to exist during EEG spikes is not absolute in the strict sense of the word, unit discharges apparently not affected by the epileptic activation were rather exceptional and represented a minority in the behavior of the various thousands of units studied. For this reason, it is believed that "hypersynchrony" of action is definitely an outstanding feature of the EEG epileptiform discharge.

EPILEPTOGENIC LESIONS FOUND IN CAT WITH AUDITORY CORTEX TUMOR

An epileptogenic lesion induced by a meningioma in the auditory cortex of a cat was found to have been capable of producing

physiological disturbances during the animal's life. Of particular interest was the finding of spontaneous changes in the activity of the epileptogenic lesion similar to those encountered in man.

The present report by Drs. O. Gonzalez Monteagudo and D. P. Purpura, NINDB grantees at Columbia University College of Physicians and Surgeons, may be considered unique and of particular interest in view of the lack of reports on histologically verified intracranial neoplasms in the cat. Their findings were published in The Cornell Veterinarian.

During the course of the study, when bilateral craniectomy was performed, a mass attached to the dura was found compressing an area of the cortex on both sides of the posterior ectosylvian sulcus. The tumor was readily removed without trauma to the underlying cortex, and the entire area was explored electrographically. Focal paroxysmal discharges were clearly distinguishable from surface potentials evoked by auditory stimuli (hand claps).

The investigators point out that meningiomas frequently produce epileptogenic lesions in the human cortex, and state that in view of the electrographic evidence in this study, it is established that an intracranial tumor involving the meninges can also induce epileptogenic activity in the cerebral cortex of the cat.

NEUROPHARMACOLOGY

DELAYED THERAPY IN METHANOL POISONING IN MONKEYS PROVIDES CLUE TO TREAT- MENT IN HUMANS

Methanol poisoning (by ingestion of wood alcohol) has been effectively treated in animals with ethanol therapy even though this

treatment was delayed as long as 12 hours. When treatment was withheld beyond this period death could not be prevented, but was delayed. This finding adds important knowledge to the treatment of human methanol poisoning.

A continuing study of methanol poisoning is being conducted by Drs. Anita Peek Gilger and Albert M. Potts and Irene S. Farkas, M.A., NINDB grantees at Western Reserve University and University Hospitals of Cleveland, Cleveland. The present phase of this study was reported in the American Journal of Ophthalmology.

The clinical course of acute methanol poisoning in monkeys resembles that of human poisoning with eye and brain damage, peripheral vasomotor shock, eventual coma, and death.

The authors describe three facets of methanol poisoning--narcosis due to the methanol itself, metabolic acidosis, and specific nervous system involvement. The latter two are presumably due to a toxic oxidation product of methanol--either formaldehyde or a formaldehyde complex. Treatment with ethanol is recommended because ethanol successfully competes with methanol for alcohol oxidation, thereby slowing down the oxidation of methanol into this toxic product.

Single oral doses of methanol were given to monkeys and ethanol therapy started at varying intervals after the poisoning. Monkeys surviving these experiments were allowed to recuperate for a minimum of three weeks and then given the same dose of methanol without treatment. Of nine untreated monkeys, seven died. Ethanol treatment could be withheld as long as 8 to 12 hours. After 16-20 hours delay the monkeys died, but treatment did delay death.

On the basis of the present investigation, the authors have outlined a recommended treatment schedule in human methanol poisoning which may prove beneficial in the prevention of serious permanent damage to a patient.

VERTIGO EFFECTIVELY TREATED WITH ANTI- COAGULANTS

Anticoagulants have been found effective in the treatment of vertigo due either to impending thrombosis of the posterior inferior cerebellar artery or to recurrent basilar insufficiency. Their use, however, is difficult and sometimes hazardous and a good response to early treatment is indicated before long-term anticoagulant therapy is used.

An evaluation of vertigo and its treatment by Dr. Fred Plum, NINDB grantee, Head, Neurology Division, University of Washington School of Medicine, appeared in Postgraduate Medicine.

In this study, prothrombin levels were kept between 20 and 30 percent of normal and the prothrombin checked weekly. Anticoagulation was maintained for one to two weeks following the subsidence of all symptoms and then cautiously discontinued. Cessation of administration of anticoagulants was not followed by further neurological damage. These observations corroborate similar findings reported by two other NINDB grantees--Dr. C.W. Millikan, Mayo Clinic, and Dr. C. M. Fisher, Harvard.

Vertigo, a clearly defined symptom resulting from acute dysfunction of the peripheral labyrinths or their direct peripheral and central nervous system connection, has been found to be the single commonest symptom in those cases in which basilar artery thrombosis has developed subsequently. In diagnosing the patient with vertigo, however, the presence of nonspecific dizziness or temporary suspension of consciousness (syncope) must first be ruled out by the patient's medical history. It is the perception of rotation which makes vertigo a distinctive symptom. And the direction of spinning identifies the site of the lesion. Most vertigo results from unilateral irritative lesions. Consequently, the sense of spinning is usually toward the opposite side of the head from the lesion.

Meniere's disease, acute labyrinthitis, tumors of the cerebellum, pontine angle, and vascular insufficiency of the basilar artery are sometimes confused with one another as causes of acute or recurrent vertigo. There are characteristic signs, symptoms, and laboratory findings which help to distinguish these diseases.

CEREBROVASCULAR DISEASES

NEURONAL DAMAGE BY X-RAY
IN RATS BELIEVED DUE TO
VASCULAR INVOLVEMENT

Neuronal damage from low energy
X-ray irradiation may be the
indirect result of a primary
interference with the blood

supply. Similar studies of irradiation with highly energized particles have resulted in the destruction of neurons with a sparing of blood vessels.

The present experiment in which large doses of low energy X-rays were used on a limited area of the brains of adult rats was conducted by Dr. M. W. Brightman of the NINDB Laboratory of Neuroanatomical Sciences and his findings were reported in Experimental Neurology.

Trypan blue spread throughout the irradiated regions, supporting the observation of an extensive breakdown of selective vascular permeability as a consequence of X-ray irradiation. The most striking early effects in the present study were characterized by a swath of petechiae throughout the lesion and by cell injury and death in the severely affected zones. Significantly, the medial, less damaged zones contained extravasated red blood cells dispersed among morphologically normal neurons and neuroglia cells. Thus, disruption of the endothelium preceded visible changes in any part of the nerve cell. The X-ray lesion was characterized by the presence of perivascular, extracellular, globular masses, 2 to 20 microns in diameter. These globules are probably derived from the contents or walls of the injured vessels and contain glycoprotein as demonstrated histo-chemically.

Most of the rats survived for 1 to 3 weeks following irradiation. During this time the water intake and urine output of the animals decreased gradually and finally dropped to zero levels. They developed a marked reduction in spontaneous movement. When the animals were prodded into activity, their movements were ataxic. Blindness developed in a number of rats. There was a considerable decrease in body temperature as these changes became evident.

In the present experiments, irradiation of the hypothalamus resulted in a dramatic metabolic upset and death within a few weeks, whereas the same amount of irradiation of an equal volume of tissue in more dorsal and anterior parts of the cerebrum was unaccompanied by any gross metabolic disturbance. Furthermore, the type of cell irradiated influences the outcome. The extent of the reaction also was influenced by the type of X-ray irradiation, the timing of the exposure, and the total volume of brain irradiated.

ELECTROENCEPHALOGRAPHIC EXAMINATION FOUND BENE- FICIAL IN DIAGNOSING CAROTID ARTERY OCCLUSION

Abnormal electroencephalographic findings in suspected internal carotid artery occlusions, when considered with blood pressure and other clinical evaluations,

may often eliminate the need for arteriography in diagnosing this disorder. Arteriography, usually regarded as a most reliable diagnostic method, is known to carry some risk to the patient.

Dr. Fletcher McDowell, Dr. Charles E. Wells, and Carol Ehlers of Cornell Medical Center, New York, studied the course of 20 cases diagnosed as suspected internal carotid artery occlusion. Their findings were reported in Neurology.

In all 20 patients, the electroencephalograms were considered to be abnormal. The most prominent electroencephalographic abnormalities were the presence of diffuse low amplitude slow wave activity and the presence of superimposed focal higher amplitude slow wave activity. In 19 of the patients, the diffuse slowing brain wave activity was usually confined to the side of the lesion, but at times the slowing involved both hemispheres. Perhaps the most striking abnormality was the exhibition of prominent focal slow wave which stood out quite clearly from the diffuse low amplitude slowing of the background activity. The severity of the electroencephalographic changes appeared to bear little relation to the severity of the clinical symptoms.

There was the usual slow improvement in these patients while the electroencephalogram remained markedly abnormal for weeks or months. One patient still demonstrated abnormalities after six years following the onset of his symptoms.

The authors state that these abnormalities, while consistent within the group studied are probably not specific for internal carotid occlusion since they might also appear in other varieties of occlusive vascular disease. However, the encephalographic record should improve with time in internal carotid occlusion which is not usually the case with cerebral neoplasm.

NINDB STUDY OUTLINES PROBLEMS INHERENT IN LONG-TERM ANTI- COAGULANT THERAPY

Problems and complications resulting from anticoagulant therapy in patients with cerebrovascular disease show

the necessity of selective administration and careful regulation of anticoagulants during the period of therapy.

A detailed study of 108 patients routinely admitted to a busy city hospital was reported in Neurology by Drs. Sigmund N. Groch, L. J. Hurwitz, Ellen McDevitt, and Irving S. Wright, of Bellevue Hospital. The study is part of a continuing investigation of cerebrovascular diseases supported by NINDB. The report concerns an evaluation of complications of therapy, and does not deal with its effectiveness.

During 27 months, 108 patients admitted to Bellevue Hospital with a diagnosis of cerebral thrombosis or cerebral embolism received anticoagulants. Of these, 61 were selected to receive treatment as part of the controlled study. In the remaining cases, therapy was considered obligatory by reason of concomitant myocardial infarction or other systemic factor. The majority of patients were over 50 years of age.

Hemorrhagic complications occurred in 20 percent of all patients treated. In 54 patients therapy was discontinued at or prior to discharge from the hospital. In 14 of these, therapy was discontinued as a direct result of complications of anticoagulant therapy. The incidence of complications was, however, significantly greater in the group where therapy was considered obligatory. In 18 patients followed for more than a year, a hemorrhagic complication necessitating discontinuance of therapy occurred in only one.

It was found that the older the patient, the greater was the incidence of complications. Most older patients also had difficulty in attending weekly outpatient clinics regularly, although eight patients over 60 years of age were able to attend for over a year. In low income groups such as represented by this series, the inability of many patients to attend the clinic constitutes a definite hindrance to assessing the value of long-term therapy.

In the outpatient, risks of anticoagulant therapy may stem from ordinary hazards of living, such as accidents where decreased coagulability of the blood is dangerous. Other disadvantages of the treatment are the risks involved in abrupt discontinuance. Two cases are described demonstrating the so-called "rebound phenomena," in which excessive clotting occurs up to six weeks after therapy is discontinued. The possibility of cerebral hemorrhage in these patients is an important factor to be considered.

The authors conclude that approximately 33 percent of all patients with strokes will be suitable candidates for anticoagulant therapy. While the use of anticoagulants for cerebrovascular disease is certainly feasible, it is found to involve definite risks and problems.

DISORDERS OF VISION

CATARACTS INDUCED BY
TOXIC AGENT REVEAL
NEW FINDINGS

Experimental cataracts produced by a toxic agent (mimosine) displayed the same early depression of mitotic activity observed in

other cataracts artificially induced but the degeneration of the lens epithelium in this experiment was much more rapid and extensive.

In an experiment by Dr. Ludwig von Sallmann, Chief of the Ophthalmology Branch, NINDB, and his associates, mimosine was used which had been isolated from the seeds of *Leucaena glauca* in crystalline form. The results were reported at the International Congress of Ophthalmology at Brussels, as part of a Symposium on the Etiology of Cataract.

The rats which had been fed mimosine displayed involvement of the cornea and conjunctival vasculature first. Shortly after, before lens opacities were visible, inflammatory iris changes of hemorrhagic character were noticed. Three days after mimosine feeding was begun histologic examination indicated the lens epithelium underwent degenerative changes, particularly in the germinative zone. Cataract development was thought, therefore, to be a primary effect of the toxic agent and not caused indirectly by other tissue injury.

Of particular interest were changes in the ultrastructure of the cytoplasm such as swelling of mitochondria and distention of the rough surfaced endoplasmic reticulum resulting in a system of tubular formations of considerable diameter. The author concludes that these morphological alterations may be the correlate of the increase in cell size described as the initial microscopic lesion of the germinative epithelium.

The investigators were unable to explain a regression of corneal edema and inflammatory or vascular signs in the anterior segment of the eye discovered after the 5th day of the mimosine diet. However, with extensive damage of the lens epithelium, fiber destruction follows and the cataract continued to completion even though the agent had lost its toxic action on other implicated tissues.

COLOR BLINDNESS STUDIED
BY ELECTRORETINOGRAPHY

A color deficiency of the retina may be the cause of color blindness. Electroretin-

ographic examinations which measure responses from the retina reveal different sensitivity curves in color-blind individuals than those which appear in normal individuals. This indicates that the hereditary types of color deficiency may originate in the retina rather than in the brain or optic pathways.

The studies of Drs. Richard M. Copenhaver and Ralph D. Gunkel, from NINDB's Ophthalmology Branch, confirm this theory and reveal additional criteria for diagnosis of color-blind persons. Their findings were reported at the Eastern Section Meeting of the Association for Research in Ophthalmology.

Congenital color blindness, more prevalent in men than in women, is not just an interesting novelty. The defect is, in some cases, a serious handicap in the selection of an occupation, as well as in the appreciation of one's environment.

Using mixtures of the three physiological primary colors--red, blue and green--individuals with normal color vision are able to match all the colors in the spectrum. These persons are said to have trichomatic vision. Many color-deficient individuals need only a mixture of two colors to match any given spectral color as it appears to them. Protanopes are individuals who are able to match all spectral colors by mixing blue and green in the right proportions, but are insensitive to red. Deuteranopes, according to previous studies, are able to match all colors by mixing red and blue and are normally sensitive to green, although unable to distinguish between red and green. Some color defectives--anomalous trichomats--use the three primary colors, but do not make normal matches.

In the tests conducted on approximately twenty-five NIH volunteers, the cornea was anesthetized, two ground electrodes were placed on the forehead and a reference electrode on the bridge of the nose. A contact lens placed on the cornea served as the counter electrode, while a beam of colored light focused on the eye acted as the stimulus. A rotating sector disc interrupted the beam at the rate of 32 times per second, eliciting electrical responses from the retina. These responses were amplified and recorded by a Grass electro-encephalograph. From these tracings, spectral sensitivity curves were calculated which revealed the extent of the abnormality in these individuals.

The results of the electroretinography tests--a test which appears to be related to what the eye sees as determined by subjective examination of the individuals--revealed that the protanopes had an increased peripheral blue sensitivity which may have replaced the red sensitivity. These findings also indicated that the deuteranopes who possess green photosensitive pigment nevertheless had a deficient green sensitivity. According to the investigators, this may be due to an interruption of "green impulses" within the retina. Another theory suggested by these findings is that there may be two types of deuteranopes--one with decreased green sensitivity and one who mixes "red and green impulses" and hence cannot distinguish red from green.

OPHTHALMIC DISORDER NOTED IN MULTIPLE MYELOMA

An understanding of corneal
crystan-like deposits rarely
present in patients with

multiple myeloma may be important in early diagnosis of this disease. The appearance, composition and diagnostic value of these crystals in the ocular tissues of children with cystinosis is well known, but the nature and significance of similar deposits in patients with multiple myeloma needs further clarification.

Dr. Samuel B. Aronson, NINDB Ophthalmology Branch, and Dr. Richard Shaw, National Cancer Institute, report the occurrence of crystalline deposits in a patient five years before a diagnosis of multiple myeloma was established. Their findings were reported in the A.M.A. Archives of Ophthalmology.

The case history reported concerns a 60 year-old woman in whom a diagnosis of bilateral non-familial crystalline corneal dystrophy was made during a routine examination at another hospital in 1952. Following this, the patient suffered an attack of iritis of the right eye and later conjunctivitis. The patient was admitted to NIH in 1957 with a diagnosis of multiple myeloma based on the results of bone marrow studies and serum electrophoresis. She was seen by the Ophthalmology Branch during her hospitalization with a complaint of itching and burning sensations of the right eye. There were no symptoms of conjunctivitis present. The cornea appeared clear and had normal luster and normal sensitivity to touch. Under the biomicroscope, masses of very fine iridescent crystalline deposits were seen interspersed through the corneal stroma and were densely packed in the anterior half of this tissue. The conjunctiva contained similar, less densely arranged particles.

Examinations during the following year showed no change in the distribution and characteristics of the iridescent crystals, nor did the patient complain of eye discomfort and the visual acuity remained normal. Histochemical studies on biopsy material from the conjunctiva strongly suggest that the crystals are cholesterol. Noteworthy is the presence of crystals in the pathologic plasma cells of the bone marrow.

In an attempt to estimate the incidence of corneal change in multiple myeloma, 13 patients with this diagnosis have since been examined biomicroscopically. None of these patients exhibited evidence of crystalline deposits in the cornea and conjunctiva. In view of the small number of reported myeloma cases with corneal crystals, it must be assumed that these deposits occur rarely, but may be regarded as a part of the clinical picture.

The findings in this patient showed similarity to those cases reported by other scientists although none were detected for as long a period prior to definite diagnosis of the systemic disease.

**NEW CONGENITAL EYE DISORDER
FOUND IN NORTH CAROLINA**

A new congenital eye disease has been found to be prevalent in an isolated area of

North Carolina. It manifests itself by the presence of gelatinous patches at the junction of the cornea and conjunctiva (perilimbal bulbar conjunctiva) and is associated with comparable lesions of the mouth mucous membrane. Without proven exception, the disorder has been found to be hereditary.

This disease entity was studied by Drs. Ludwig von Sallmann and David Paton of NINDB's Ophthalmology Branch and Dr. Carl Witkop of the National Dental Institute in 109 patients of all age groups. The results of the eye examinations were reported at the annual meeting of the American Ophthalmological Society.

All patients studied are family members of a large tri-racial isolated population in Halifax County, North Carolina. They are called Halowar Indians and are of Indian, Negro and Caucasian racial origin. Interbreeding is common, and several additional congenital anomalies such as retinitis pigmentosa and a diffuse neurological disorder have been observed in members of the group. They are indigent tobacco and cotton farmers who live in small wooden shacks and subsist on a diet of root vegetables, beans, some canned foods, and meat when available. The general nutritional state of these people is poor, but as a rule physical examinations did not reveal vitamin deficiency disease.

Basic similarities of the eye lesions were observed in this family clan. The changes were bilateral and congenital and consisted of variously arranged semitranslucent proliferations which histologically show epithelial dyskeratosis. These vegetations varied from disfiguring visible lesions (pinguecula-size) to tiny depositions that are seen only with magnification. The lesions have a rather firm consistency and cannot be scraped off.

During routine physical examinations of the ten patients actually admitted to the Institute (Clinical Center) for further study, it was found that each of the seven having the familial disease also had lesions in the mouth. In the 109 cases examined, thirty patients ranging in age from two to sixty-eight were found to have the congenital eye lesion and four others were suspected of having the disease. Fifteen persons from

the same socio-economic environment, but unrelated to the patients under study were also examined and none had the eye lesions.

The Halowars call their disease "red eye" because of the redness due to the eye lesions. Some patients stated the lesions had been present throughout their life, but would come and go. Others gave a history of seasonal variation in the severity of the disease. Occasional patients said they had a "skin which grows across the eye" but eventually peeled off. One six-year old girl was examined in which the latter condition was found to be true and a corneal membrane was readily peeled off with forceps. Only a few cases examined to date have had definite corneal changes attributable to the disease.

Further examination of the family clan has continued and of approximately 200 additional members of this genealogy, 44 cases of eye lesions have been reported. All those with eye lesions have also been found to have the oral lesions and no cases of definite oral lesions have been found without evidence of ocular changes. Pathological material from both the eyes and the mouth shows histological similarities. The extent of the histopathologic changes vary from patient to patient, as does the severity of the clinical picture of individuals.

NINDB GRANTEE PROPOSES NEW THEORY FOR EARLY GLAUCOMA THERAPY

A study of primary open-angle glaucoma suggests the necessity of lowering the intraocular pressure of the eye more than previously thought necessary. "Open-angle" glaucoma is a common but not easily recognized form of primary glaucoma. The new theory implies that the intraocular pressure should be lowered sufficiently to prevent trabecular damage rather than merely enough to prevent damage to the optic nerve. There is evidence that the trabecular meshwork is a pressure-regulating mechanism which if damaged is less able to control the changes in intraocular pressure caused by variations in aqueous outflow.

The findings of Dr. Milton Flocks, NINDB grantee at Stanford University, appeared in the American Journal of Ophthalmology. The report describes in detail the histologic findings in 16 cases of primary open-angle glaucoma and 11 cases of secondary glaucoma.

There is a large familial factor in the incidence of open-angle glaucoma. Dr. Flocks' new theory suggests that persons

destined to have it have relatively vulnerable trabeculae which are injured by levels of intraocular pressure which ordinarily would not damage the trabeculae or optic nerve. When the curve of intraocular pressure reaches the point where trabecular degeneration has occurred to cause insufficiency of the pressure-regulating mechanism, progressive glaucoma may result unless treatment is begun.

The study revealed marked degenerative changes in the meshwork and Schlemm's canal, beginning in the external portion of the trabecular meshwork. The location and character of the changes indicate that they are responsible for the decreased facility of aqueous outflow characteristic of primary open-angle glaucoma.

Only a small number of eyes with early open-angle glaucoma have been described histologically to date, because of the difficulty of obtaining specimens before certain changes have occurred. Further investigations will attempt to determine whether prolonged elevation of intraocular pressure in animals will produce these degenerative lesions of the meshwork.

EXPERIMENTAL CHORIORETINAL BURNS INDUCED IN ANIMALS BY INDIRECT OPHTHALMOSCOPY

A new instrument for causing experimental chorioretinal burns in the living eye has been developed. This type

of light coagulation of the human retina and choroid offers a simple and efficient method of treatment of retinal lesions.

NINDB grantees of the Retina Foundation of Harvard Medical School - Robert J. Brockhurst, M.D., Ernst Wolf, Ph.D., and C.L. Schepens, M.D. - describe the new instrument and some preliminary results obtained with it in the A.M.A. Archives of Ophthalmology.

Previously, light coagulation therapy has been effective only to the limits of direct ophthalmoscopy. In view of the many advantages of indirect over direct ophthalmoscopy in locating fundus lesions, especially in the peripheral portions of the fundus, an instrument was developed for light coagulation utilizing indirect ophthalmoscopy. The instrument not only permits more rapid and accurate localization of lesions to be treated, but also permits more adequate treatment of lesions located anterior to the equator. In this area, where most retinal breaks are found, a non-surgical approach to the treatment of retinal breaks without detachment is most often applicable.

The authors have developed an apparatus which utilizes a mercury-vapor-arc light and indirect ophthalmoscopic control of the burning light. In order to obtain chorioretinal burns, the light must be brought to a sharp focus on the choroid and retina. When the light is accurately focused and directed at the area of the fundus to be treated, the shutter is opened to expose the fundus to the full intensity of the mercury-vapor lamp. Experimental chorioretinal burns were produced in pigmented adult rabbits and guinea pigs. After five to seven days, pigmentation began to appear and finally the lesion resembled a typical chorioretinal scar as seen in chorioretinitis due to diathermy applications. Histologically the area then showed a well-demarcated chorioretinal adhesion.

Since the focal length of the human eye is more than two times that of the rabbit eye, the intensity of the light reaching the human retina is less than one-half of that which reaches the rabbit retina. The present equipment has produced a chorioretinal burn, but a fairly long exposure to the light was necessary. Further experimental work is now in progress to improve the light output of the instrument so that effective and rapid burns may be obtained in human eyes.

DISORDERS OF HEARING

"WALTZING" GUINEA PIGS USED IN EAR STUDIES

In recent animal studies on the ear, it was found that the fibrous layer (stria vascularis), which provides the blood supply to the cochlea, also generates strong electricity even in totally deaf animals. Potential activity has previously been believed to be confined to the sensory cells of the inner ear.

Drs. I. Tasaki and C. S. Spyropoulos, NINDB's Laboratory of Neurophysiology, conducted experiments on deaf guinea pigs using normal guinea pigs as controls to determine the anatomical localization of the source of steady potential activity within the cochlea. Their findings were reported in the Journal of Neurophysiology.

The deaf animals - called "waltzing" guinea pigs because of their lack of equilibrium function - are a special breed developed at NIH. Although these animals are born with a normal cochlea, the cochlea begins to degenerate approximately two weeks after birth. About four months of age, the Corti organ of the cochlea disappears. The animals show no outer ear reflex movement to loud auditory stimuli and no signs of microphonic response.

The cochlea in the special breed was exposed and a recording microelectrode was inserted into the endolymphatic space through the basilar membrane. There was a negative variable potential in the space above this membrane. On further advancement of the microelectrode into the stria vascularis, there was a sudden appearance of strong positivity at the electrode tip. The blood vessels in this area were found to be almost intact. Marginal and other cells were also present, but appeared to be slightly atrophic.

In the normal animals, the potential was found to rise even more sharply when the recording electrode was moved toward the middle of the stria vascularis and to return to zero level when it was moved away. There was strong positive potential in the cochlea when the recording electrodes were placed in the proper position.

According to the investigators, similar conclusions as to the source of endocochlear potential were reached simultaneously by other scientists. However, in this investigation it was believed that the special breed provided better experimental data since more concrete evidence was provided than in experiments in which the sensory cells were artificially destroyed.

NEW THEORY OF AUDITORY NERVE IMPULSE INITIATION PRESENTED

For the first time, a theory suggests a mechanism whereby nerve impulses in individual auditory nerve fibers are initiated in

mechano-receptors, such as the cochlea. The report of the study describes the process of anodal polarization by which hair cells of the cochlea may be made especially responsive to physical stimuli.

The results of the investigation were presented by Dr. Ichiji Tasaki, Head, Section on Special Senses, NINDB, at a Conference on Neural Mechanisms of the Auditory and Vestibular Systems. It is a preliminary report of the hypothesis on the mechanism of biological transducers. The new data, developed recently in Dr. Tasaki's Section, is derived from investigations of the general properties of the excitable membrane.

Research has already shown that nerve or muscle becomes inexcitable when it is immersed in potassium-rich media. The hair-bearing ends of hair cells are, in fact, immersed in a medium which contains a high concentration of potassium. If the hair-bearing ends are rendered inexcitable by this high potassium content in the scala media (cochlea), it would not be possible for the hair cells to respond to sounds.

Dr. Tasaki believes, however, that the microphonic responses do arise at this end of the hair cells, because the phase of microphonics reverses at the moment the tip of the recording micro-electrode crosses the reticular lamina. This end of the hair cells, he reports, must have a high sensitivity to mechanical stimuli.

It has been known for some time in medical research that a region of nerve rendered inexcitable by a local application of potassium chloride can be made excitable again by anodal polarization. The most significant point in these investigations, according to Dr. Tasaki, is that when the cell membrane immersed in potassium-rich media is tranversed by an inward-directed current of a certain intensity, the membrane becomes extremely unstable. This phenomenon is regarded by the investigator as being a consequence of the existence of two stable chemical configurations in the membrane.

In the cochlea, the hair-bearing ends of the hair cells are exposed to anodal polarization by virtue of the existence of a large potential difference between the endolymph and the perilymph (fluids of the inner ear). This potential in the endolymphatic space is essential for maintenance of a high sensitivity of the hair cells to sound stimuli. An increase in the polarization at the hair-bearing end of the hair cells enhances the amplitude of the action potential of the whole nerve and the microphonics.

In conclusion, Dr. Tasaki states that a critically polarized membrane in potassium-rich media is expected to undergo occasional spontaneous transitions between the two stable configurations. This can be regarded as the mechanism whereby spontaneous discharges are evoked in sensitive mechano-receptors.

MINAMATA DISEASE

CONSUMPTION OF CONTAMINATED
FISH BELIEVED RESPONSIBLE
FOR UNUSUAL NEUROLOGICAL
DISORDER IN JAPAN

Minamata disease, an obscure neurological illness which assumed epidemic proportions on one of the main Japanese islands several years ago,

was probably caused by consumption of contaminated fish.

Drs. Douglas McAlpine and Shukuro Araki, NINDB grantees, Department of Neuropsychiatry, Kumamoto University Medical School, Kyushu, Japan, reported their findings in The Lancet.

The disease, named for the town of Minamata, where it was particularly prevalent, was first recognized in 1953. Within two years it had caused death in more than a third of its victims and serious disability in most of its survivors.

Evidence in the search for the cause of the disorder points to contaminated shell fish and other marine life caught in Minamata Bay and used as daily food. It is suspected that the contamination was caused by industrial waste. Until 1950, the effluent from a nearby fertilizer factory containing numerous chemical compounds, was diverted into the open sea by a channel. At that time, however, the channel was closed and a new one constructed which ran directly into Minamata Bay. About 1951 a new vinyl plastic processing plant was also opened at the factory.

The neurological features of the disease appeared similar to those present in toxic states due to certain metals. In all the adults affected, there developed numbness in the extremities, unsteady gait, serious speech difficulty and increasing disability. In the more severely affected there developed confusion, psychotic reactions and severe intellectual deterioration. Most of the 52 patients studied, ten of whom were children, also suffered disturbances of hearing and vision.

Since a ban was placed on fishing in the bay in late 1956, the number of new cases of the disease has decreased appreciably. Research at Kumamoto University is continuing in the hope of discovering the nature of the toxic agent responsible for this unusual outbreak.

APPLICATION OF NEW TECHNIQUES

NERVE ACTIVITY MEASURED BY
ELECTRONIC ELECTRODE

Electrical potentials, at an
inaccessible point inside the
axis of a nerve which is

electrically and physically remote from external electrodes, may now be reliably measured by an improved electronic technique.

Dr. John W. Moore, NINDB Laboratory of Biophysics, reported the results of experiments on nerve activity evaluation at a meeting

of the Institute of Radio Engineers. This technique has been called an "electronic electrode" because the use of feedback made possible the equivalent of a good contact at a remote point, similar to that accomplished by inserting physical electrodes.

In myelinated nerves, widely found among vertebrates, there are very small active patches, called "nodes" about 2 millimeters apart, where the heavy insulation over the nerve is drastically reduced. Several approaches have been used previously to try to obtain measurements of the potential across this active membrane, but have had limited accuracy or have required the use of very difficult techniques in the preparation and experimental stages.

One of the factors limiting the accuracy in previous methods was the high internal longitudinal resistance of about 50 million ohms (unit of electrical resistance) between these nodes. The technique as used by this investigator is an electronic negative feedback to increase, in effect, the external resistance by a large factor. This method makes the resistance of the measuring circuit large compared to the internodal resistance and thereby assures high accuracy. This system is similar to and elaborates on the scheme developed by British, Swiss and Swedish investigators.

With such an electronic nulling technique used in the present experiments, it was possible to measure, with speed and accuracy, resting potentials, action potentials and conductivity changes across a nodal membrane. This technical achievement provides another tool to aid in bioelectric research and will be tried in the study of other nerve preparations.

PROGRAM HIGHLIGHTS IN THE FIELD OF NEUROLOGY AND SENSORY DISORDERS--1959

During 1959 the National Institute of Neurological Diseases and Blindness moved further toward conquest of the many baffling neurological ailments which comprise the third cause of death in the United States. The Institute has recruited and integrated highly specialized personnel in the Institute at Bethesda; it is attacking the shortage of scientific investigators by providing grants for training programs of several kinds.

Increasing effectiveness of joint activities with non-Governmental medical research is demonstrated by such collaborative projects as a five-year study for early detection of that major cause of blindness, glaucoma. Another, and larger, collaborative study on wastage in human pregnancy moved from the pretest into the action stage.

The Institute's national and international attack on neurological disorders is outlined below:

COLLABORATIVE AND COOPERATIVE STUDIES

GLAUCOMA SUBJECT OF COLLABORATIVE STUDY

A five-year study to evaluate methods for screening and diagnosing glaucoma began

June 1, 1959. Techniques currently applied to the detection and identification of glaucoma are now being evaluated at four research centers through grants awarded by NINDB at a cost of approximately \$115,000 a year.

The grants were awarded to the Wilmer Institute, Johns Hopkins University Hospital, Baltimore; Moffitt Eye Hospital, University of California Medical School, San Francisco; Department of Ophthalmology, Washington University School of Medicine, St. Louis; and the Department of Ophthalmology, State University of Iowa, Iowa City.

Plans for the evaluation study were developed by the Chronic Disease Program, Bureau of State Services, PHS; in cooperation with NINDB and a statistical analysis of the data obtained from the study will be made by the Chronic Disease Program.

PROGRESS IN THE COLLABORATIVE PROJECT FOR CEREBRAL PALSY AND MENTAL RETARDATION

The largest of various research programs relating to cerebral palsy, mental retardation and other defects in children is

the Institute's Collaborative Study which officially began January 1959 after 2½ years of intensive preparation. It is the first large-scale effort to collect information on all factors that might conceivably have a bearing on cerebral palsy and other neurological disorders and the first research program ever undertaken to collect and analyze this information before rather than after such disorders develop.

In January, "final" study forms, covering the various aspects (prenatal, labor, delivery, neonatal, etc.) were distributed to each of the 17 participating institutions. As the year progressed and as each institution became confident that procedures and forms had improved sufficiently, the hospitals were permitted to change from pretest to "final" study numbering systems.

As of October, some 5500 mothers and 4200 babies have been processed in the pretest series, and approximately 3300 mothers and 1800 babies have been processed under the "final" study series.

NINDB TRAINING GRANTS

TRAINING PROGRAMS IN NEURO- RADIOLOGY INAUGURATED BY NINDB

Postgraduate training grants in neuroradiology have been awarded for the first time.

The awards were given to the Albert Einstein College of Medicine and College of Physicians and Surgeons of Columbia University, both of New York, in the amount of approximately \$50,000 for the first year. The programs to begin July 1, 1960, will mark the addition of another important phase in the development of post-graduate research training for clinicians, similar to the establishment of organized training on a program basis in neuropathology and pediatric neurology.

CONFERENCES

CONFERENCE ON AUDITORY AND VESTIBULAR SYSTEMS AT NIH

A two-day conference on Neural Mechanisms of the Auditory and Vestibular Systems was held at

NIH in June, 1959. The conference, sponsored by NINDB, and organized

by Dr. Grant L. Rasmussen, was introduced by Dr. Richard L. Masland, Director, NINDB (at that time, Assistant Director). Dr. William F. Windle, Chief of NINDB Laboratory of Neuroanatomical Sciences, was the presiding officer.

The conference was attended by outstanding medical scientists from research centers in this country, Norway and Sweden, and England. Papers were presented on recent studies and general discussions concluded each day's meetings.

CONFERENCE ON POSTGRADUATE TRAINING IN OTOLARYNGOLOGY

The Otolaryngology Postgraduate Training Committee of NINDB held its First Program Director's

Conference, May 14, 1959, to review the status of training of investigators in otolaryngology.

The Committee also took up formulation of recommendations for the further development and improvement in the training of such personnel. The administrative, basic and clinical aspects of the training program were the subjects of these recommendations.

PUBLICATIONS

PROGRESS IN NEUROBIOLOGY OUTLINED IN PUBLICATION

A published monograph entitled "The Biology of Myelin" has resulted from an earlier NINDB-sponsored conference on "Progress in Neurobiology." The authors of this symposium--biochemists, biophysicists, neuropathologists, biologists, and clinicians--present for the first time current knowledge of the structure of myelin, its chemical and cellular make-up, its development, and some of its alterations in disease.

Among the specific topics discussed in detail are the ultra-structure of nerve myelin, major chemical changes occurring during myelinization, the effect of peripheral nerve degeneration and regeneration on the myelin sheath, and genetic influences affecting the constitution and maintenance of the myelin sheath.

The book is edited by Dr. Saul Korey, NINDB grantee at the Albert Einstein College of Medicine.

NEW BROCHURE ON "LITTLE STROKES" PUBLISHED

A new booklet entitled "Little Strokes--Hope Through Research" tells the story of stroke patients who have survived their attacks to live long, useful lives. It urges a plan of action: careful diagnosis, proper treatment and rehabilitation, and an intelligent attitude by family and patient.

The booklet reviews research currently being conducted by NINDB and through its grant-supported non-Federal hospitals and medical centers. Listed as Public Health Service Publication No. 689, copies may be obtained from the National Institute of Neurological Diseases and Blindness, Bethesda 14, Md. or the Government Printing Office.

INTERNATIONAL COLLABORATION

SOVIET SCIENTISTS VISIT NINDB

Three Soviet scientists arrived in Washington in February to make a 30-day survey of U. S. research developments in physiology and pharmacology of the nervous system. They spent two days at NINDB before visiting research centers throughout the country.

An American neurological mission of six scientists went to the USSR late in 1958 to make a similar study and conferred with the Soviet delegation during their stay in this country.

The members of the group were: Dr. Sergey Viktorovich Anichov, Professor, Head of the Department of Pharmacology, Sanitary-Hygiene Medical Institute at Leningrad, and spokesman for pharmacology achievements in the USSR; Dr. Vladimir Sergeyevich Rusinov, Head, Department of Physiology and Pathology of the Nervous System, Institute of Neurosurgery of the USSR Academy of Medical Sciences; and Dr. Vasil'y Vasil'yevich Zakusov, Director of the Institute of Pharmacology and Chemotherapy of the USSR Academy of Medical Sciences.

The Soviet scientists visited this country under the provisions of a January 1958 agreement between the U. S. and the Union of Soviet Socialist Republics to exchange missions in eight different

fields of medical sciences: New antibiotics, microbiology, physiology and pharmacology of the nervous system, biochemistry, radiobiology, metabolic diseases, endocrinology and community and industrial hygiene.

**THREE INTERNATIONALLY KNOWN
INVESTIGATORS JOIN NINDB
STAFF AS VISITING SCIENTISTS**

Professors Ulrich Franck of Germany and Torsten Teorell of Sweden participated for three months in intensive, detailed

investigations made by NINDB's Section on Special Senses in an attempt to explain the basic processes of excitation. These processes not only provide the elementary activity of the nervous system, but also are related to many other biological phenomena such as muscle contraction, cell division, amoeboid movement and receptor activity. It has now been shown that this activity in living cells has many characteristics which resemble those of certain non-biological electrochemical and physiochemical systems. Professors Franck and Teorell are specialists in these systems.

Professor Ulrich Franck is Chairman of Electrochemistry at Edward-Zintl-Institut, Darmstadt, Germany. Professor Torsten Teorell is Chairman of Physiology and Biophysics, Uppsala University, Uppsala, Sweden.

Dr. William A. H. Rushton, who joined NINDB's Ophthalmology Branch for one year, is recognized for his contributions to electrophysiology. His recent studies on the physical measurement of rhodopsin in the normal living eye and the eye of color defectives have provided a new, ingenious approach to the understanding of color vision and its abnormalities. Dr. Rushton is Director of Medical Studies, Trinity College, Cambridge, England.

**MULTIPLE SCLEROSIS
CONFERENCE IN COPENHAGEN**

A Geomedical Conference under the auspices of the World Federation of Neurology Commission

on Biometry and Genetics, Dr. L. T. Kurland, NINDB, Chairman, was held at the "Domus Medica" in Copenhagen, June, 1959. The conference was sponsored by the WFN, the National Institute of Neurological Diseases and Blindness, and the Danish Multiple Sclerosis Society.

The principal theme of the conference concerned studies of the frequency of multiple sclerosis in various geographic areas of the world and the evaluation of current epidemiological techniques used in geographic neurology.

ENCEPHALITIS SYMPOSIUM
SPONSORED BY THE WORLD
FEDERATION OF NEUROLOGY

More than fifty scientists from fourteen countries met in Antwerp, Belgium in May, 1959, to discuss the neuropathology and pathophysiology of the various encephalitides occurring around the world. Among the countries represented were Austria, Belgium, Czechoslovakia, Denmark, France, Romania, Switzerland, and the United States.

The Symposium was organized by Dr. Ludo van Bogaert, President of the World Federation of Neurology, and Director of the Institute Bunge in Antwerp and was sponsored by the World Federation of Neurology, Centre Interuniversitaire de Recherches Neuro-pathologiques, and the National Institute of Neurological Diseases and Blindness.

FIRST INTERNATIONAL GRANT
IN NEUROLOGY AWARDED BY
NINDB

Two grants totaling \$138,207 were awarded the World Federation of Neurology, with headquarters in Antwerp, Belgium, by the NINDB in February, 1959. These were the first Government-supported grants in neurology to an international organization.

One grant of \$126,190 will provide partial support for a small central staff in Belgium with consulting medical scientists from many countries. Universities and research centers throughout the world are being encouraged to cooperate in such international studies as perinatal morbidity and cerebrovascular diseases. The remaining grant of \$12,017 was used for a symposium on encephalitis held in Antwerp in May, 1959. The conferees classified and defined the known types of encephalitides so that a common language is provided for the exchange of information among researchers throughout the world.

The World Federation of Neurology, an international organization, is composed of delegates from the leading neurological societies of 33 countries, including the United States.

Dr. Charles M. Poser, Assistant Professor of Medicine and Head of the Section of Experimental Neurology, University of Kansas, has been appointed medical executive officer of the World Federation of Neurology to work with Dr. Ludo van Bogaert, President of the Federation, in the development of this program of international neurological research.

HIGHLIGHTS OF PROGRESS
IN RESEARCH AT THE
CLINICAL CENTER

1959

Items of Interest on Program Developments and Research
Studies Conducted and Supported by the Clinical Center

USE OF CLOSED CIRCUIT TV IN MEDICAL RESEARCH

The vast increase in scope and intensity of biological and medical research during the past 10 years has provided revolutionary new concepts in the causation, methods of treatment and prevention of many diseases. As a direct consequence the need for accelerating the dissemination of information concerning these new developments has become increasingly important.

The National Institutes of Health has been making significant progress in helping to meet this challenge through its television activities which are administered by the Clinical Center. The scope of these activities is broad, embracing the exploration of the value of television-related electronic techniques in our research environment as well as the study of its application to education and communication, and the operation of "WNIH-TV-The Clinical Center System."

This useful tool in scientific education and communication, biologic research and patient care came into being after eight years of intensive studies, planning, and development. In 1955 a series of practical tests were reported in the film "Electronic Image Processing," a term coined by NIH staff and now found in the scientific literature as the generic word for the use of scanning techniques in biologic research. The period 1956 through 1958 was devoted to installation of the Center's uniquely flexible television distribution system. During fiscal years '57 and '58 research and development of the project continued. Finally, beginning in July 1958 activities have been focused on the education and communications aspects.

The medium of TV has proved useful in solving or alleviating a number of problems at NIH. For example, increased numbers of professional and pre-professional visitors have been scheduled in large groups whenever possible. In this way organized presentations could be arranged, with television substituting for laboratory and patient area tours. The use of TV has also proved to be of value in increasing the effectiveness of scientific presentations at medical and scientific meetings, and to care for overflow audiences.

In the past 12 months 18 programs utilizing TV were presented to audiences totalling 3,555 persons. Approximately 2,350 visitors have been given a first-hand view of NIH at work. On one occasion 370 high school science teachers were gathered in the Clinical Center auditorium. They were toured, via TV, through the National Cancer Institute Laboratory of Chemical Pharmacology and the General Medicine Branch's Leukemia Studies Nursing Unit. Questionnaires returned by the teachers indicated almost unanimous approval of the program. More important, an analysis of the relative time involved in this method of presentation vs. the personal handling of visitors showed that the same result was accomplished in one day that otherwise would have required 3 full weeks of scientists' time.

Indication of the full value of the Clinical Center system was realized at the time that a visit from the Chairman of the Council of the Supreme Soviet was scheduled. Since protocol required that the visitor be toured through patient and laboratory areas where the tremendous press retinue could not be permitted, arrangements were made to follow the tour through the Clinical Center on large-screen closed circuit TV in the auditorium.

Kinescope records have been made of several live television presentations so that a permanent film record of current research activities will be available for future programs for professional and pre-professional visitors. During 1959 approximately 1,000 persons viewed kinescope films at the Clinical Center. The viewers included professors of preventive medicine who are members of Medical Education for National Defense, groups of high school science teachers, medical students, and Public Health Service Commissioned Officers.

One TV demonstration achieved a dual purpose. Viewed by 70 members of the Institute for Advancement of Medical Communication, the unusual technique of simultaneous pickup and projection to a large audience was of interest. This group was composed of representatives from medical professional

organizations, medical schools, foundations, broadcasting firms, national voluntary health organizations, and the pharmaceutical industry. Viewing the program at the same time, a group of 250 science students found the subject matter interesting from an educational standpoint.

PROGRESS OF NEW CLINICAL CENTER SURGICAL WING

Surgery is achieving a uniquely important role in modern medicine and in certain types of medical research. This has been brought about by the rapid advances in surgical techniques and instrumentation. To fully utilize newer developments, it became apparent that more comprehensive facilities were needed at the Clinical Center. During the past two years intensive studies and long planning by many consultants were concluded and construction of a new surgical wing began. Construction is now 20 percent completed.

Scheduled for occupancy early in 1961, the structure will introduce innovations in design of facilities for neurosurgery and cardiac surgery. The building itself, a circular free-standing structure, will accommodate the newest instruments and further insure the safety, effectiveness, and efficiency that surgeons require. Flexibility of floor plan will also provide space for an increasing number and complexity of recording apparatus and increasing numbers of specialists and technicians engaged in the research surgical procedures and around the operating table.

Consideration has been given to such crucial problems as the flow and quality of air in the operating room, so important in neurosurgery, particularly, where one deals with vital tissues that are utterly destroyed by infection; to methods of lighting; improved techniques of electronic shielding; to the larger number of persons involved in complex surgical procedures necessitating the adaptation of flexible intercommunications arrangements; to the usefulness of television (close-range and long-range) for instructional purposes; systemic recording; and photography. Special facilities are also being provided for the Center's modern Blood Bank.

This new operating wing will enable the Clinical Center to provide an entirely different contribution in the conduct of research practice. Evidence of this is already available from the many visits which have been made to the Clinical Center during the year by hospital architects, administrators, and surgeons who wish to study our designs in order to incorporate many of these ideas in their plans for future construction projects.

SAFER AND MORE EFFECTIVE ANESTHESIA

In addition to the primary function of providing necessary diagnostic and other essential services to the clinical research programs of NIH, Clinical Center departments frequently have an opportunity to conduct research or to collaborate with the Institutes in the trial of new therapeutic materials and devices. An example of this is the work of the Anesthesiology Department of the Clinical Center in the following studies which are making a contribution toward achieving safe and additionally effective anesthesia.

In cooperation with the National Institute of Dental Research, studies on general anesthesia on dental outpatients are being conducted with methohexital, a new short-acting barbiturate developed by the Eli Lilly Company and produced by them under the trade-name, Brevital. As part of this same study, hexacyclium, a new synthetic drug with parasympatholytic actions, is being compared with atropine and scopolamine in regard to its effects in suppressing salivary secretions. Continuous physiologic monitoring of these patients includes electrocardiographic and electroencephalographic recordings. Recently, arterial oxygen saturation as determined by a Woods-Waters ear oximeter has been observed during anesthesia and surgery.

Methohexital has proved to be a more potent barbiturate than Sodium Pentothal and Sodium Methitural and the recovery period is appreciably shorter. The lack of confusion and "hangover" effect are noteworthy, but a possible disadvantage is its tendency to produce tachycardia. Sodium Pentothal, produced by Abbott Laboratories, and Sodium Methitural are widely known and have been studied here and elsewhere during the past three years. Methitural was formerly known by the generic name, methiturate, and is commercially available through the Schering Corporation under the trade-name, Neraval.

Hexacyclium has proved to be superior in suppressing salivary secretions. Its action is apparent within 3 minutes following the intravenous injection of a 2 mgm. dose in adult patients, and its use has elicited no subjective complaints. When given alone, it fails to produce a pulse rate increase as do atropine or scopolamine, although it does have a tendency to cause tachycardia. Hexacyclium is widely used in the treatment of peptic ulcer and is produced by Abbott Laboratories under the trade-name, Tral.

In cooperation with the neurosurgical group of the National Institute of Neurological Diseases and Blindness, a study of the electroencephalographic effects of methohexital is being conducted to evaluate the feasibility of its use on patients undergoing selective temporal lobe resection for epilepsy. This new drug may fill the need for a short-acting anesthetic for use during the injection of a local anesthetic agent into the scalp and for removal of the bone flap.

Previously available anesthetic agents of adequate potency have caused enough changes in the electroencephalogram to hamper studies done on the exposed brain of the awake patient, which are necessary for accurate localization of epileptogenic foci. The short action of methohexital, together with the fact that it appears to activate rather than depress epileptogenic foci, favors its use under these circumstances.

PRESERVATION OF STAPHYLOCOCCAL BACTERIOPHAGES

One of the most severe problems to arise in recent years in the field of hospital care has been the development of strains of staphylococcus that are resistant to presently available antibiotics. It is a matter of grave concern to hospitals in this country to find methods of prevention and control of this problem of infection.

Workers in the Clinical Pathology Department of the Clinical Center have developed what is believed to be the first practical method for preserving staphylococcal bacteriophages.

Hitherto, the average hospital has not been able to employ phage typing as a control measure because of the cost and difficulty of continuously maintaining cultures of the many types of unstable staphylococcal bacteriophages. Using the new method, biological product manufacturers or other large central laboratories now could supply preserved bacteriophages to hospitals where they may be stored until needed.

When an outbreak occurs, staphylococci from patients can be cultured by standard methods and challenged with preserved phage that has been reconstituted. The type or types of phage which destroy sections of the culture identifies the strain of staphylococcus. Similar identification can then be done on staphylococci obtained from hospital employees, physicians, other patients, and fomites such as dust, instruments, or other suspected sources.

The procedure uses a modification of the standard agar plate method of propagation of bacteriophages. Each bacteriophage is allowed to infect its host staphylococcus which results in enormous multiplication of the bacteriophage and destruction of the host staphylococcus. The suspension of bacteriophage, after removal of any remaining viable bacteria, is mixed with double strength skim milk and placed in small glass vials. The mixture is frozen, dried and the vial heat-sealed while under vacuum. It may be kept refrigerated for at least one year.

Reconstitution of the phage is accomplished by adding an appropriate amount of trypticase soy broth to the vial's contents to provide the desired dilution of phage.

First described at a Staff Conference of NIH scientists and guest physicians, the method was developed over the past 2 years by Charles H. Zierdt, bacteriologist in the Clinical Pathology Department of the Clinical Center. This work was reported in The American Journal of Clinical Pathology.

HIGHLIGHTS OF PROGRESS

IN RESEARCH ON

BIOLOGICS

1959

Items of Interest on Program Developments and Research Studies Conducted and Supported by the Division of Biologics Standards

The administration of those provisions of the Public Health Service Act which pertain to the control of biological products in interstate and foreign commerce is the primary responsibility of the Division. This responsibility is executed by the issuance of two types of licenses--establishment and product--for the production and sale of such products, following a determination by the Division that the prescribed standards for safety, purity, and potency have been met. At the end of the calendar year, 180 establishment licenses and 1,247 product licenses were in effect, the latter covering 282 specific products. In the first eleven months of 1959, tests of 3,409 individual lots of various biological products were made, ranging from relatively simple sterility tests to potency determinations involving both animal and tissue culture tests. Of equal importance in ensuring the continued safety, purity, and potency of these products is the preparation and distribution of physical reference standards to manufacturers and laboratories engaged in biological standardization throughout the world. Approximately 4,000 vials of such standard preparations are distributed each year by the Division.

Through the Division's expanding research, which is a vital part of its program, continuous efforts are made to improve and to develop new testing procedures, as well as to improve methods of preserving and storing physical reference standards, to correlate them with standards established in other countries, and to develop new ones.

COMPARATIVE NEUROVIRULENCE FOR RHESUS MONKEYS OF LIVE POLIOVIRUS STRAINS

The criteria of attenuation of live poliovirus, as recommended by the World Health Organization Expert

Committee in 1957, are based on the reduction of neurovirulence for monkeys. In fact, the strains administered to hundreds of thousands

of persons in Africa, Asia, Europe, and Latin America, to several millions in the USSR, and to small groups in the United States during recent field trials, were selected on this basis. In an effort to obtain comparative information under standardized conditions concerning the neurovirulence of these strains for monkeys, DBS studied the three sets of Type I, II, and III poliovirus strains used in worldwide field trials.

Each of the strains was tested by intraspinal and intracerebral inoculation in groups of monkeys of the same species, using identical methods and techniques both in the inoculation of the animals and in the interpretation of the results. The Division's safety testing program for inactivated poliomyelitis vaccine, which over the past four years has involved the use of 20,000 monkeys, provided invaluable experience in inoculation methods as well as in interpretation of the lesions seen in the histopathologic sections of the monkeys' central nervous system.

The data from the studies, presented by Dr. Roderick Murray, Director, DBS, and Dr. Ruth Kirschstein, DBS pathologist, at the World Health Organization- and Pan American Health Organization-sponsored meeting, clearly showed that all of the strains tested by intraspinal inoculation produced lesions in the spinal cord in varying degrees. When inoculated in an amount necessary to consistently infect human beings by the oral route, all three Type I viruses produced paralysis in monkeys. The Sabin Type II strain showed less tendency to spread to the cervical and brain stem of the spinal cord than the Lederle and Koprowski Type II strains. Titers (virus count) of all three Type III strains were comparable but, again, the Sabin strain showed less tendency to spread.

In the intracerebral inoculations by the intrathalamic route, no lesions were found in the lumbar, cervical, or brain stem sections with the Sabin strains. With the Koprowski strains, lesions were seen with Types I and III, but not with Type II.

The Public Health Service Committee on Live Poliovirus Vaccines, in its first report, stated that "the relationship of such laboratory findings to potential pathogenicity for human beings after oral administration must be resolved before live poliovirus vaccines can be accepted as safe and standardized public health procedures or licensing of particular strains can be recommended."

INCREASED AMOUNT OF POLIOMYELITIS ANTIGEN IN INITIAL DOSE SHOWS RAPID DEVELOPMENT OF ANTIBODIES

The feasibility of evoking early antibody response in nonimmune persons by the inoculation of large amounts

of poliomyelitis antigen is being investigated by DBS virologists Dr. Samuel Baron and co-workers.

The first study showed that a single 10-ml inoculation of poliomyelitis vaccine results in earlier development of antibody than the initial 1-ml inoculation of the prescribed immunization series. These observations were made in a limited group of nonimmune persons who were inoculated with vaccine somewhat above the median potency of commercial vaccine available at that time (New England Journal of Medicine).

The most recent study was undertaken to determine the effect of vaccines containing acceptable but lower amounts of antigen when given in a single 10-ml dose to nonimmune persons, as well as to gain further experience regarding the safety of administering large amounts of antigen in a single dose. In this study, 133 children--none of whom gave a history of having received poliomyelitis vaccine--each received a single inoculation of vaccine of low but acceptable potency: 45 received 1 ml; 45 received 3 ml; and 43 received 10 ml. Prior to inoculation, antibody determinations were made by the metabolic inhibition test, and 46 percent of the group were found to be triple negative, i.e., to have no antibodies to any of the three polioviruses (A.M.A. Journal of Diseases of Children).

As in the earlier study, the antibody response was shown to be dependent on the amount of antigen inoculated. A 10-ml dose of low potency vaccine resulted in earlier evidence of neutralizing antibody levels than did a 1-ml dose of the same vaccine. Based on the criterion that the presence of any measurable antibody is presumptive evidence of protection against paralysis, 47 percent of the triple-negative children who received the 10-ml dose were "protected" from Type I paralytic poliomyelitis.

No untoward effects were observed in the 43 children of the second study or in the 69 persons of the previous study who received 10-ml inoculations of vaccine.

These data indicate that, while single 1-ml inoculations of some vaccines would offer relatively low levels of protection, single 10-ml inoculations of minimum potency vaccine could be expected to protect about half of the nonimmune persons, and single 10-ml inoculations of median-to-high potency vaccine would give virtually 100 percent protection. Thus, in the event of epidemic conditions, a single 10-ml inoculation of poliomyelitis vaccine could be an effective means of rapid protection for nonimmune persons. If vaccine potency continues to increase, however, a 1-ml inoculation may eventually give as much protection as the 10-ml dose given in this study.

**POTENCY OF POLIOMYELITIS VACCINE
SHOWS CONTINUOUS RISE DURING
1959**

The continuing rise in potency of poliomyelitis vaccine produced in this country during 1959 was

reported by Dr. Roderick Murray, Director, DBS, at the 5th International Meeting of Biological Standardization this fall. The meeting, sponsored by the International Association of the Societies for Microbiology, was held in Israel.

The data presented were based on a survey of vaccines released by the Public Health Service since April 1955. The lots of vaccine were divided into groups according to date of release, with each group representing vaccine released within a two-month period. The median potency of vaccines within such groups, as measured by the monkey potency test, has shown a continued and sustained rise with respect to Type II and Type III components, beginning in early 1958 and continuing through June 1959 (the latest period for which data were available). With Type I component, the rise was apparent in 1959 only, however, when the figures were also computed as medians expressed in terms of doses, it was apparent that the rise in potency of Type I occurred in some lots as early as November 1958.

The DBS data were presented as part of a review of potency as well as methods of potency determination of vaccine produced during the past 18 months. A similar presentation was made of the vaccine produced prior to 1957 at the 4th International Poliomyelitis Conference in Geneva in 1957. At that time, the median potency of vaccine being produced although still within the acceptable range, was low. This was particularly evident for Type I and III components.

Data on the chick potency test, which has been used experimentally as an additional test by vaccine producers in this country as well as by DBS, were also presented by Dr. Murray. The chick test has not been officially adopted because the monkey test is functioning in a much more satisfactory manner since the general level of vaccine potency has increased. The correlation between the two tests is the subject of continuing study.

Other potency tests which have been considered are the complement-fixation tests and the antibody combining test. The latter is still under study with a view to determining its utility for routine use. DBS scientists believe, however, that final potency requirements of the vaccine should be measured in terms of an animal test.

SIMULTANEOUS IMMUNIZATION AGAINST FOUR DISEASES NOW POSSIBLE IN INFANCY

Multiple antigen preparations containing diphtheria toxoid, tetanus toxoid, and pertussis

vaccine have been in use in this country for a number of years and have wide acceptance in pediatric practice. This year, a combined product containing poliomyelitis vaccine in addition to the three commonly used antigens has been licensed for commercial use. The combined antigens are designed only for administration to young children, for whom it substantially reduces the number of injections necessary heretofore. Administration to older children and adults is inadvisable because of the well-known hypersensitive reaction which results from the use of diphtheria toxoid in full dosage in this age group.

The preparation of the quadruple antigen has been a complex process. The compatibility of the four components and their stability in combination were subjected to several years' study by the manufacturers prior to clinical trials to test the antibody response in children.

The multiple antigen dosage schedule consists of an initial series of three doses given one month apart. This is necessary in order to meet the immunization characteristics of all four components of the product. A fourth injection should be given six months to a year later. Thus when such a product is used, the child receives in effect a fourth booster dose of poliomyelitis vaccine.

IMMUNIZATION OF ADULT FEMALE HAMSTERS PROTECTS OFFSPRING AGAINST VIRUS-INDUCED TUMORS

In previously reported studies, Drs. Bernice Eddy, DBS, and Sarah Stewart, NCI, have repeatedly shown that

sarcomas can be induced in hamsters inoculated with the SE polyoma virus at birth and up to 21 days of age. Generally, with increased age at time of inoculation, decreased susceptibility and increased latent periods are evidenced in hamsters. These investigators have also shown that formation of circulating antibodies can be stimulated by the virus in mice, hamsters, and rabbits, and that hamsters and mice that live long enough after virus-induced tumors have developed also have circulating antibodies.

In a recent study, it was shown that maternal antibody may be responsible for the reduction in the number of tumors in the offspring and in the delay of their appearance. In this respect the polyoma virus behaves as do many other viruses in that maternal antibodies are protective to a certain extent when the young animal is most susceptible and does not possess its own defensive antibodies.

In this study, one group of adult female hamsters was injected with antigen prepared from polyoma virus on the first, fourth, or seventh day of pregnancy; another group was not immunized. The offspring of both groups were given 0.2 ml. each of undiluted polyoma virus subcutaneously when 1 to 3 days of age. Fifty-three (96%) of the 55 offspring of the nonimmunized mothers developed multiple tumors and died within 45 days after injection. Only 29 (38%) of the 76 offspring of the immunized mothers developed tumors.

This work was reported at the annual meeting of the Federation of American Societies for Experimental Biology by Dr. Bernice Eddy, DBS.

TUMORS IN RATS INDUCED BY
POLYOMA VIRUS TISSUE CULTURE
PREPARATION

A method of inducing neoplasms
in randomly bred rats by
inoculation with SE polyoma
virus propagated in mouse

embryo tissue cultures was reported by Dr. Bernice Eddy, DBS, and Dr. Sarah Stewart, NCI, in their collaborative research with the SE polyoma virus.

Earlier work with the tumor-inducing agent, which Drs. Stewart and Eddy discovered about three years ago, indicates that fluids from tissue culture preparations inoculated with SE polyoma virus induce tumors in hybrid mice, randomly bred Swiss mice, and hamsters.

In a study (Eddy, Stewart, et al, Journal of the National Cancer Institute) 65 newborn Sprague-Dawley rats (a breed of rats in which the occurrence of spontaneous renal sarcomas is less than 1%) were inoculated with SE polyoma virus that had been propagated in mouse embryo tissue cultures. Eighteen of the rats developed renal sarcomas and seven developed subcutaneous tumors. A tissue mince of renal sarcoma and spleen from a rat with an induced kidney tumor was used as an initial inoculum for a series of mouse embryo tissue cultures. The culture fluid from the fourth serial passage induced the same array of visceral tumors in hamsters (lungs, liver, heart, gastrointestinal tract) as those produced by previous tissue culture lines of the SE polyoma virus. The twelfth serial passage again induced renal sarcomas in rats. The other tissue passages were not tested. The rat is the third mammalian species to develop significant numbers of tumors after receiving, in the newborn period, SE polyoma virus grown in tissue culture.

For other reports on SE polyoma virus see NCI's "Highlights of Progress on Cancer Research."

VIRUS GROWTH OF CELL CULTURES MAINTAINED WITH SKIM MILK MEDIUM

The development of
serum-free medium
containing skim milk

(Baron and Low, Science, 1958) which effectively maintains a wide variety of cell cultures led to a study of viral sensitivity of various cell cultures in this medium.

Comparative titrations of 22 different virus strains, performed by Dr. Samuel Baron and co-workers, showed that the infectivity titers of poliomyelitis, ECHO, Coxsackie, influenza, herpes, measles, and vaccinia viruses in the presence of skim milk medium were equal to or greater than those obtained when established maintenance medium was used.

Strains of adenovirus maintained in skim milk showed a lower infectivity for the cells than in established maintenance media. Experiments to determine whether the skim milk acts directly to repress adenovirus multiplication or whether it lacks the cell-mediated enhancing factor of other media indicate that its inhibitory action was probably an effect on the tissue culture cells rather than an antiviral effect. Similar cell-mediated effects have been observed by other investigators.

The overall results of the study indicate that the medium is free of inhibitor for infectivity and permits full sensitivity to infection, thus confirming the suitability of skim milk as a maintenance medium. (Gochenour and Baron, Proc. Soc. Exper. Biol. & Med.)

RELATION OF MYXOMA DESOXYRIBONUCLEIC ACID (DNA) TO FIBROMA-MYXOMA VIRUS TRANSFORMATION

The transformation of
fibroma to myxoma provides
a model for the study of
the biological activity of

nucleic acid in animal viruses. Myxoma virus heated at 65° C. for periods well beyond those needed to destroy infectivity was first used two years ago to establish this tissue culture reaction (Kilham, Proc. Soc. Exper. Biol. & Med., 1957). This original transforming agent, TAM, was assumed to be a myxoma particle with a denatured outer protein coat.

The aim of the recent study was to remove or alter the outer coat of the virus in order to expose the inner core of desoxyribonucleic acid. With the aid of Dr. Joseph Shack, NCI chemist, Dr. Lawrence Kilham, Laboratory of Bacterial Products, DBS, showed that this transforming agent, TAM, is basically desoxyribonucleic acid.

This demonstration is dependent upon the use of specific enzymes. Whereas DNase destroyed 100 percent of the transforming activity of the chemically treated TAM, RNase had no destructive action whatever. TAM prepared with urea treatment has been found to have no loss of activity, as compared with the heat-treated TAM.

Whether the particles of UREA-TAM are without a protein coat, or whether the DNase sensitivity results from formation of crevices which permit approach of the enzyme to the nucleic acid has not yet been determined. One consideration suggests that the final product is not completely free DNA--this is that the activity of UREA-TAM is comparable to that of TAM itself, whereas the viral activity of nucleic acids derived from viruses is generally very low.

The work was reported by Dr. Kilham at the annual meeting of the Federation of American Societies for Experimental Biology.

L FORMS OF BACTERIA POSE PROBLEM IN TISSUE CULTURE WORK

The conversion of bacterial
contaminants, despite
antibiotic therapy, to
variants known as L forms,

is of growing concern to laboratories working with tissue culture cell lines. In many ways, the L forms of bacteria resemble the pleuropneumonia-like organisms called PPLO, which also grow without a rigid cell wall. The contamination of tissue cultures used in research in the DBS Laboratory of Bacterial Products have led to an investigation by Dr. Michael Barile and co-workers. L forms of bacteria have been isolated from twelve tissue culture lines, although in repeated examination neither L forms nor PPLO were isolated from the primary monkey and rabbit kidney cells used, nor from the tissue culture media or medium supplements.

Several bacteria which were developed from L forms isolated from the Lac cell line were induced to revert to L forms when grown in tissue culture media containing penicillin and streptomycin. It appears probable that the L forms in the cell lines were induced by the action of antibodies on bacteria.

Study of these intracellular organisms by DBS researchers thus far has shown that growth curves of the L form and the L-form-infected viable tissue culture cell parallel each other. The population of the organism remains approximately 100-fold greater than the cell and maintains its population as long as the cell remains viable.

Since it appears that antibiotics cannot be relied on to control completely the bacterial contamination in tissue culture lines, further study is planned to determine 1) whether antibiotics can

induce bacteria to produce L forms in tissue cultures; 2) the effect of chemical and physical factors, including antibiotics, on clearing tissue lines of L forms; and 3) the effect of L forms on the infectivity of certain viruses in tissue culture lines.

DETECTION OF HEMOLYTIC DISEASE OF THE NEWBORN

A five-month collaborative study designed to yield information for use in

establishing regulations and standards for control testing of anti-human serum has been completed by Dr. J. T. Tripp, Laboratory of Blood and Blood Products, DBS, and Dr. Sol Haberman, Department of Immunohematology, Baylor University.

In the past, difficulties have been encountered when anti-human serum has been used for laboratory diagnosis of hemolytic disease of the newborn (erythroblastosis) due to sensitization by antibodies of the ABO system. Although anti-human serum is effective in detecting almost all cases of this disease caused by Rh immunization, it has generally been found to be valueless in the detection of those cases produced by ABO sensitization. Baylor University Hospital was selected to collaborate in this work because of its interest in the detection of this problem and because of the clinical material it could provide.

The reactions of several anti-human serums with bloods from the newborn having clinical or latent ABO erythroblastosis were compared with a control series of newborn infants without ABO sensitization. The data indicate that serums selected according to their reactions within the ABO system, rather than solely by reactions with Rh antibodies, are reliable for detecting ABO erythroblastosis. This offers a possibility for the development of standards applicable to products of this nature.

SENSITIZED RED CELLS SUCCESSFULLY STORED IN FROZEN STATE FOR A YEAR

Sensitized red cells, which occur through contact with red cell antibodies resulting

from transfusion or from pregnancy, are usually detected by the Coombs' test. Correct grouping and typing of such cells is difficult however, because of their polyagglutinability in albumin typing serum and the necessity of using the indirect Coombs' technique for antigen determination. The advantage of storing sensitized red cells to act as a reference for comparison with the erythrocytes of the patient who no longer manifests a positive direct Coombs' test led Dr. Sherwin V. Kevy and Mrs. E. Morrison, Laboratory of Blood and Blood Products, DBS, to study the stability after frozen storage of an in vivo antigen antibody bonding on red cells.

Sensitized red cells were obtained from four patients with hemolytic disease of the newborn, three with acquired hemolytic anemia, and one with chronic lymphatic leukemia. The cells were washed three times in large volumes of saline to remove free antibodies, then treated with glycerol solutions and placed in deep freeze at -20° or -40° C. The stored cells were thawed at 37° C. and deglycerolized. Eluates were prepared from the cell stroma. It was shown that sensitized cells can be stored with only slight decrease in the strength of the positive direct Coombs' test and without alteration in the specificity of the eluate.

Antibody specificity is of more than academic interest. Identification of the antibody serves as a diagnostic tool by which valuable information may be obtained regarding transformation therapy. For example, since acquired hemolytic anemia presents a variable course, it would be valuable to obtain, during various stages of the disease, red cells which can be preserved in glycerol for subsequent auto-transfusion. This would serve both as a method of evaluating therapy and for delineating the pathogenesis of the disease process.

Previous studies by DBS scientists have demonstrated that storage in glycerol at subzero temperatures preserves the antigenic activity and viability of the erythrocytes for both auto- and hemo-transfusion. This recent study demonstrates that storage in glycerol at subzero temperatures will not disrupt the antigen-antibody bond formed in vivo.

This work was reported in the American Journal of Clinical Pathology.

COMPARATIVE STUDY OF ULTRAVIOLET INACTIVATION OF ANIMAL VIRUSES

A study to determine whether
the ultraviolet light
inactivation characteristics

of animal viruses would be useful in classifying known viruses and identifying unknown viruses was undertaken by Dr. Samuel Baron and co-workers, Laboratory of Viral Products.

Aqueous suspensions of various animal viruses were exposed to ultraviolet radiation. Samples were taken at appropriate intervals and assayed for infectivity. The decline of infectivity as related to the dose of irradiation was then plotted for each virus. The experiment was controlled by simultaneous inactivation of the Saukett strain of poliovirus which has a highly stable inactivation rate.

The ultraviolet inactivation rate of all three poliovirus types was found to be similar. The rates of the ECHO, Coxsackie, and EMC viruses, which are similar in size and in other respects to poliovirus, were indistinguishable from that of poliovirus. The inactivation rate of ECHO 10, which is not only larger than the other ECHO viruses but has other distinguishing properties as well, was slower than poliovirus, Coxsackie, EMC, and the other ECHO viruses.

If a correlation existed between the inactivation rate and the size of animal viruses, as has been shown to be true of bacteriophage, then viruses larger than ECHO 10 would be expected to inactivate more slowly. However, Dr. Baron found the opposite to be true. LCM and fibroma viruses, which are larger than ECHO 10 and have other distinguishing properties, inactivated more rapidly.

These findings indicate that there is no relationship between UV inactivation rates and the size of animal viruses. Neither does the nucleic acid content appear to be the sole determinant of sensitivity to ultraviolet light since some of these viruses have the same nucleic acid content and entirely different inactivation rates. There is however a pronounced similarity in the inactivation curves of those viruses which fall within the currently accepted classifications of viruses. For example, polioviruses 1, 2, and 3; ECHO viruses 1, 4, 7, 8, 12, and 13; and Coxsackie viruses A9 and B3 had similar inactivation curves. Human adenovirus 3 and monkey adenovirus SV-1, although of different origin, had similar inactivation curves which were specific for adenovirus. Inactivation curves for four influenza viruses were similar to one another and also type specific.

The study indicates that animal viruses which bear a broad relationship to one another have similar UV light inactivation properties. This correlation is based on the supposition that the sensitive areas of related viruses, as well as the intensity of ultraviolet radiation on these areas, are of the same order of magnitude. Further study of the potential application of this correlation as an aid in virus classification and identification is being made. This work was reported by Dr. Baron at the annual meeting of the Federation of American Societies for Experimental Biology.

PHOTODYNAMIC METHOD OF INACTIVATING VIRUSES STUDIED

The inactivation of viruses
by irradiation with visible
light in the presence of a

photosensitizing dye is being investigated as a potential method in the preparation of vaccines. Although the photodynamic action of a dye on protozoa was known as early as 1898 when Raab reported that paramecia were killed when exposed to visible light in the presence of methylene blue dye, the technique has not been widely applied.

The possible use of this phenomenon in methods of preparing vaccines prompted Drs. C. W. Hiatt and Jerome J. Helprin (Journal of Bacteriology) to study the inactivating effect of ordinary visible light on various viruses in the presence of trace amounts of toluidine blue dye. Bacteriophage, a virus which attacks bacteria, was used as a model in this particular study because of the high concentration of the virus which may be obtained and the precision of its assay.

A small amount of toluidine blue dye was mixed with a suspension of bacteriophage and exposed in a thin layer to light from an incandescent bulb. The dye absorbs the light energy at a specific wave length, and since the target point of this energy is apparently the nucleic acid of the virus the antigenic protein of the virus remains unaffected, although the reproductivity of the virus is destroyed. In studies using two different phages, it has been demonstrated that there is a wide differential in rate of uptake and tenacity of binding of the photosensitizing dye.

This selective method of inactivation, if it could be applied to other viruses, would obviate some of the physical limitations of current inactivation methods, in which the margin between inactivation of the virus and destruction of its antigenic properties is narrow. The technique also has a potential use in the classification of viruses, since some appear to be susceptible to this system and others do not. Vaccinia virus, for example, is readily inactivated by this method, while poliovirus is rather resistant.

PROGRESS IN MEASLES VACCINE RESEARCH

In April 1958, NIH virologists, independent investigators, and interested manufacturers met

with DBS staff to confer on problems relating to the development of a measles vaccine. The general agreement at that time for the need of a vaccine that would prevent measles and its often severe complications led to the current studies in progress at a number of centers, including Harvard Medical School and the Universities of Maryland, Colorado, and North Carolina.

In November of this year a second meeting was called by Dr. Roderick Murray, Director, DBS, to review the progress and to consider the problems that have developed in measles vaccine research since the first meeting.

The principal investigators working under three-year NIH grants are Dr. Edward C. Curnen, Professor and Chairman of the Department of Pediatrics, University of North Carolina School of Medicine; Dr. C. Henry Kempe, Professor and Head of the Department of Pediatrics,

University of Colorado Medical Center; and Dr. Fred R. McCrumb, Jr., Assistant Professor of Medicine and Director, Section of Infectious Diseases, University of Maryland School of Medicine.

Dr. John Enders, whose work with measles virus established the basic principles for a measles vaccine, presented data obtained in his own and in Dr. Kempe's laboratory with the Enders' avianized Edmonston strain of virus. Evidence to date indicates that a relatively small dose (as expressed in tissue culture ID/50) of the Enders' vaccine injected by a parenteral route will evoke an immunologic response in susceptible children. Dr. Kempe has also carried out a limited study with a killed measles vaccine. A proposed study in a school for the deaf was outlined by Dr. Curnen who is exploring methods as well as effects of vaccination. Dr. McCrumb, who is studying the prophylactic value of both living and killed vaccines by exposure of vaccines to naturally occurring measles virus, presented some preliminary data on viremia. Drs. Frederic and Erna Gibbs, of the Department of Neurology, University of Illinois School of Medicine, were invited to present an evaluation of the encephalitic complications of measles. About one out of a thousand cases is accompanied or followed by encephalitis, which produces 20-30 percent fatalities and leaves neurological damage in about half of the survivors.

Many of the immunological aspects of a potential measles vaccine are being worked out. Special emphasis will be placed on the development of high titer material, satisfactory stability, and correlation of serological and virological methodology. The processing of reference material and the development of suggested laboratory procedures will be carried out by DBS.

HIGHLIGHTS OF PROGRESS
OF RESEARCH IN
GENERAL MEDICAL AND BIOLOGICAL SCIENCES
1959

Items of Interest on Research Studies Supported by the
Division of General Medical Sciences

It is the principal purpose of the research program of the Division of General Medical Sciences to strengthen research in the sciences basic to medicine and biology and to provide support for expanded studies in certain important health and medical problems of an applied nature.

Under the direction of the Research Grants Branch, the program is carried out entirely through grants to medical schools, universities and other non-Federal research institutions.

The basic research supported by the Division is crucially important to medicine in providing more fundamental information on man's basic physiological and biochemical processes and on the nature of life itself.

The Division also supports considerable research into the problem of man's effort to live healthfully in his increasingly complex physical environment, and supports studies in certain clinical sciences which do not come under the Institutes of NIH.

For simplicity of description the program can be grouped into four major areas, as follows:

The Chemistry of Life Processes, including the fields of physiological chemistry, metabolism, nutrition, pharmacology, microbial biochemistry, enzymology, vitaminology and protein structure.

Fundamental Genetics, Cell Biology and Human Development, including the fields of embryology, genetics, cell biology, cell differentiation, fetal physiology, fetal biochemistry, placental physiology and related areas.

Research in Public and Environmental Health, including the fields of epidemiology and biometry, diagnostic, preventive

and therapeutic measures applicable to population groups, nursing, accident prevention, food toxicology, air and water pollution, occupational medicine and radiation.

Clinical and Preclinical Research into some of the basic problems of medicine, This work includes studies in anatomy, pathology, endocrinology, anesthesiology, pediatrics, general surgery, orthopedics, obstetrics, gynecology, dermatology, and studies in the treatment of burns and shock.

Selected examples of research findings in these areas are presented under the headings of BIOCHEMISTRY, BIOLOGY, CLINICAL RESEARCH and ENVIRONMENTAL HEALTH.

BIOCHEMISTRY

DGMS GRANTEES PRODUCE
CELL-LIKE SPHERULES FROM
COPOLYMER PROTEINOIDS

In earlier work, Sidney W. Fox, of Florida State University, has reported making a primitive protein from combinations of 18

amino acids. In Science he has reported work in carrying the process a step farther by heating the proteinoid in water, resulting in the production of extensible cell-like spherules. The work is supported by grants from the Division of General Medical Sciences, from the National Science Foundation and the General Foods Corporation.

The spherules usually were of uniform microscopic size, between 1.5 and 3 microns in diameter, with variations in size and tendency to coalesce and lose shape being influenced by the addition of substances to the hot water. For example, a 0.15 percent solution of sodium chloride produced spherules of 1.4 microns diameter; a 10 percent solution produced spherules of 0.4 - 0.6 microns diameter and a 20 percent solution produced almost no spherules.

The cell-like entities retained their individual integrity for several weeks. When they were centrifuged at 2000 revolutions a minute for five minutes they retained their shape and integrity.

As a result of this work, the researchers are further investigating the concept of thermal denaturation of proteins. They theorize that in the early history of the earth the effect of boiling the proteins in water may have been to denature only the surface of the proteins, leaving the inside intact and thus forming the cell membrane.

Thus the article points out that spontaneous prebiological processes in the first cooling of the earth could have produced enormous quantities of such cell-like membranes from the proteinoid with the "likelihood that some of these entities would also enclose enough spontaneously generated biochemical apparatus to permit replication in a sterile world."

RELAXIN FOUND TO HAVE AT LEAST THREE COMPONENTS

In a paper presented before the American Society of Biological Chemists in Atlantic City, New Jersey, Edward H. Frieden, of the Arthur G. Rotch Laboratory, Boston, Massachusetts, has reported on work in analyzing relaxin.

The work is important in understanding the precise activity and significance of relaxin, which is secreted by the ovaries and apparently serves to relax the uterus during childbirth.

Previous work by this researcher has indicated that relaxin was composed of a single molecular series. Following work supported by a Division of General Medical Sciences grant, however, Dr. Frieden now has reported finding three components.

The relaxin preparations, isolated from the ovaries of pregnant sows, were separated in a solvent system of 2-butanol and 0.2% trichloroacetic acid. A total of 355 transfers were made; with the relaxin compounds spread rather uniformly over 50 tubes.

Pooled material from this distribution was rerun in the same solvent system, separating the three components. The amino acid contents of the components were determined by paper chromatography of their acid hydrolyses and were found to be qualitatively identical. They included cystine, aspartic acid, glutamic acid, glycine, serine, histidine, lysine, arginine, alanine, valine, theonine, leucine, and isoleucine.

PERFUSION STUDIES OF THE HUMAN PLACENTA YIELD NEW DATA

Dr. Philip Troen of the Department of Medicine, Harvard Medical School and Department of Medical Research and Yamins Research Laboratory, Beth Israel Hospital, Boston, Massachusetts, has studied the human placenta using perfusion techniques in an effort to obtain direct data on placental endocrine function. He has found two new effects of human chorionic gonadotropin placental metabolism. A report of his work appears in the proceedings of the conference, Recent Progress in the Endocrinology of Reproduction. The work is supported by a grant from the Division of General Medical Sciences.

In his studies, Dr. Troen obtained placentas directly after delivery from normal patients. A modified Tyrode's solution containing penicillin and streptomycin was used for perfusion. The perfusing fluid left the placenta through the umbilical arteries which were incised on the fetal surface of the placenta. The fluid was replaced every hour.

Previous demonstrations by other investigators have shown that in placental homogenates, estradiol stimulated citrate metabolism. Dr. Troen used intact placentas, comparing their responsiveness with that of placental homogenates. He found that in the intact placenta, estradiol had no effect on citrate disappearance until human chorionic gonadotropin was added. Then the rate of citrate metabolism increased significantly. The investigator considers this a new and previously undescribed action of human chorionic gonadotropin.

In another study, Dr. Troen provided experimental evidence that human chorionic gonadotropin can affect the placental metabolism of estrogen. Estriol is excreted in large quantities during pregnancy, but the source of this metabolite is not known. The results of a series of perfusion tests on the placenta indicated that under certain conditions, human chorionic gonadotropin altered the metabolism of estradiol with the formation of a new substance, thought to be estriol. The investigator has shown that this metabolite can be produced in the placenta in vitro provided the hormone human chorionic gonadotropin is added. It was previously known that estradiol could be converted to estrone by the placenta but not until Dr. Troen's perfusion studies, in which he added human chorionic gonadotropin, was it shown that estradiol could also be converted to a metabolite resembling estriol in the placenta.

Dr. Troen has also examined the problem of possible placental corticosteroid production. His data indicate that the placenta has the in vitro capacity to produce compounds with certain characteristics of corticosteroids. However, it is not possible to infer that the perfused state is indicative of the in vivo function of the placenta.

The investigator concludes that further study is required before the hormonal and nonhormonal factors affecting placental metabolism in vivo can be fully understood.

RAPID TEST DEVELOPED
FOR MEASURING INULIN
IN BLOOD AND URINE

Writing in Clinical Chemistry, Victor E. Levine, of the Department of Biological Chemistry and Nutrition of the Creighton University School of Medicine, Omaha, Nebraska, has revealed a

rapid, convenient method for determining the quantity of inulin in the blood and urine. The work was supported by a grant from the Division of General Medical Sciences.

Intravenous injections of inulin often are made on patients to determine the extent of glomerular filtration and to determine extracellular water and extracellular space. The task then is to recover the inulin rapidly and quantitatively. Usual methods often require hydrolysis and possibly the removal of glucose by fermentation with yeast.

The method revealed by Levine involves the use of vanillin, which, in the presence of sulfuric acid, forms with inulin a deep red color complex that yields a characteristic absorption spectrum with a peak at 520 millimicrons. Optical density is then determined spectrophotometrically.

In the blood test, two specimens are required, one being taken prior to the administration of inulin to serve as a control. Both bloods then are centrifuged and the plasma is deproteinized with zinc sulfate solution and sodium hydroxide, and filtered. A reagent blank, of distilled water, zinc sulfate and sodium hydroxide, is prepared, filtered and treated in the same manner as the plasma filtrate. The filtrates then are heated and cooled and optical density is read with a Beckmann spectrophotometer, model DU.

A similar procedure is followed for urine.

The quantity of inuloid material in the control plasma or in the control urine is subtracted from the inulin plus inuloid material in the inulin-containing plasma or inulin-containing urine in order to obtain the actual quantity of inulin present.

Inulin filtration measurements are valuable in kidney function and in the study of the pathologic physiology of many diseases, including cardiovascular-renal and endocrine disorders, and in the study of the effects of drugs and hormones on renal function.

ION METABOLISM STUDIED

Leonard B. Kirschner of the Department of Zoology, Washington State College, Pullman, Washington, has reported in the Archives of Biochemistry and Biophysics that adenosine has the same effect on swine erythrocytes in increasing the metabolism of the cell in vitro as on human erythrocytes. The work was carried out on grants by the State of Washington and by the Division of General Medical Sciences.

The researcher added adenosine to swine blood in storage, with the finding that the ability of the erythrocytes to extrude sodium and to maintain labile phosphate esters was definitely increased. This indicates that swine blood can be an easily-obtainable and inexpensive material for the study of ion metabolism, which study may shed light on the mechanism of this same phenomenon in human blood.

BIOLOGY

JUVENILE HORMONE OF INSECTS FOUND IN HUMAN PLACENTA

Carroll M. Williams, of Harvard University, has reported that the human placenta, rats and certain bovine organs contain a factor indistinguishable from the "juvenile hormone" previously found only in secretory glands in the neck region of most insects. The work was supported by grants from the Division of General Medical Sciences and the National Cancer Institute and reported in a recent issue of the English journal, Nature.

The hormone, which normally serves to prevent the metamorphosis of immature insects, is tested on the pupae of the silkworm, Antheraea polyphemus, in which it demonstrates its activity by blocking metamorphosis.

Williams initiated the research following findings that ether extracts of various invertebrates give positive tests for juvenile hormone and similar findings of juvenile hormone activity in aqueous extracts of bovine adrenals.

Williams' first tests were on 1-day-old rats which were anesthetized and blended at room temperature in 3:1 diethyl ether-ethanol. "After washing with water and evaporation of the solvent," the article states, "a golden oil was obtained which gave positive tests for juvenile hormone."

The next series of tests were on 50-gm samples of pulverized preparations of bovine organs. The extracts were chromatographed on alumina columns by precisely the same techniques that fractionate juvenile hormone in extracts of insect material. The individual fractions were dissolved in a small volume of peanut oil and injected into A. polyphemus pupae. Positive tests were obtained for extracts of the thymus, bone marrow, placenta, ovary, corpus luteum, adrenal cortex, liver, testis, kidney and spleen. Thymus gave extracts of the highest activity and adrenal cortex the next highest.

Further positive tests then were carried out on active fractions from fresh calf thymus and liver, deep-frozen pituitaries

of sheep, beef tenderloin and fresh human placenta. Positive tests were carried out also on purified extracts of ordinary heavy cream. Negative tests were carried out on wheatgerm oil, soy-bean oil, and dried brewer's yeast.

In concluding the article, Williams wrote: "In view of the extraordinary biological activity of this hormone on the growth, metamorphosis and aging of insects, it seems important to decide whether . . . (the) hormone may play a part in mammalian physiology or whether its presence in higher forms is something of a biochemical curiosity."

FIRST CHROMOSOME COUNT OF TERATOMA REPORTED

Dr. Michael Galton, of Harvard Medical School, Boston, Massachusetts, working under a grant

from the Division of General Medical Sciences, has reported the first chromosome count of a metastatic teratoma as a step toward solving the problem of the origin of these tumors. The work was reported in a recent issue of Lancet.

Most investigators agree that teratomas are of gametal origin. The previous finding by other investigators of two primary extragenital sex chromatin-positive teratomas in males strongly suggests that the tumors arise from parthenogenesis of a single haploid cell with chromosome reduplication. The histology and frequent presence of sex chromatin suggest the diploid chromosomal constitution of teratomas, but only actual chromosome counts can reveal the ultimate origin.

The grantee observed squashed cells in metaphase cultured from a colonic metastasis of an ovarian teratoma in a 24-year-old woman. All cells were obviously diploid. In each instance, chromosomes numbered 46, and they suggested a normal female distribution with the XX allelomorph.

These findings do not answer the question of origin, although the investigation did result in the first chromosome count of such a tumor and establishes a method for further analysis; similar studies of teratomas in males will be necessary.

CHROMOSOMAL SEX DETECTION FOUND POSSIBLE IN EXTRA-FETAL PRODUCTS

Arthur R. Sohval, of The Mount Sinai Hospital, New York, has reported in the Annals of the New York Academy of Sciences that

chromosomal sex detection is possible in the human newborn and fetus from examinations of the umbilical cord, placental tissue and fetal membranes. His work was supported by a grant from the Division of General Medical Sciences.

The work involved an extension of observations by M. S. Barr and associates that intermitotic nuclei of females contain a characteristic mass of chromatin, the sex chromatin, which is rarely present in males. Barr announced a new histological method for the diagnosis of sex utilizing a skin biopsy.

Sohval has carried out a systematic investigation of the nuclear sex chromatin in the umbilical cord, placental tissue and fetal membranes of the human newborn, and of fetuses of various ages.

Material studied included fresh specimens obtained at the time of delivery of 55 male and 50 female newborn infants, all apparently normal; sections of uterine curettings of 19 therapeutic abortions performed during the sixth to tenth week of gestation; and histological preparations of placenta and umbilical cord from 28 instances of spontaneous abortion, with fetal ages from 15 to 30 weeks.

Sex chromatin was identified in from 10 to 50 percent of the readable nuclei in the umbilical cords, placental tissue and fetal membranes associated with female fetuses. It was not encountered in any of the specimens from known males, making it evident that the chromosomal sex of extra-fetal products of conception corresponds to that of the fetus itself.

The value of the finding is found in its use in the early recognition of anomalous sex development, especially when the external genitalia are normal or when they are so inconspicuously ambiguous that the condition may be overlooked.

FACTORS CITED IN REVIVING
MICE COOLED TO
LESS THAN 1° CENTIGRADE

Dr. James A. Miller, Jr., of the Emory University School of Medicine, Emory, Georgia, has reported on means of reanimating

mice that have been subjected to extreme cold. His findings appear in the American Journal of Physiology. He carried out his experiments at the National Institute for Medical Research, Mill Hill, London, England, and the Wenner-Gren Cardiovascular Research Laboratory, Stockholm, Sweden. The work was supported by a grant from the Division of General Medical Sciences.

Only recently has it been possible to reanimate whole animals from near zero temperatures, by means of appropriate pre- and post-hypothermic treatment. In his tests, Dr. Miller employed various methods of cooling and resuscitating young adult male white mice. The general technique for cooling involved placing the mice in sealed jars in a cold room for 60 minutes, rinsing them in ice water and packing them in ice. Records of temperatures were made each minute during the periods of rapid change.

No animals were frozen. In their confinement, the mice developed gradual hypoxia and hypercapnia.

Reanimation of the mice was begun at varying intervals of from 15 to 60 minutes, and when body temperatures registered from $5^{\circ}\text{C}.$ to $0.5^{\circ}\text{C}.$ or lower. Most of those mice which were kept cool for 30 minutes or more registered temperatures of $0.2^{\circ}\text{C}.$ and occasionally $0.0^{\circ}\text{C}.$ The mice were rewarmed with a 60-watt electric light bulb. The three indices of recovery used were: the time at which blood was first seen to enter the forefeet, the first breath, and the first attempt to turn over.

The investigator wished to discover whether air, oxygen, or mixtures of oxygen and carbon dioxide were most effective in reanimating mice. The poorest results were obtained with 100% oxygen, the best with 95% + 5% carbon dioxide. This last group had the smallest number of animals with weak or paralyzed hind legs, a frequent accompaniment of hypothermia.

As an application of his studies, Dr. Miller stated that the reduction of deep body temperature has been shown to protect newborn and adult guinea pigs from lethal exposures to asphyxia. Several apneic premature human babies, after treatment with hypothermia and carbon dioxide, gained the ability to breathe. The cardiac activity of one premature baby was maintained for 21 hours by reducing temperatures to below $22^{\circ}\text{C}.$ In another case, a woman suffering from metastatic carcinoma recovered from one hour of cardiac arrest and a temperature of $9^{\circ}\text{C}.$ when artificial respiration was applied with 95% oxygen + 5% carbon dioxide.

Dr. Miller commented that means of preventing vasoconstriction seem to be the most important element in dealing with hypothermia and suggested that the presence of carbon dioxide is helpful in this respect.

SURFACE EPITHELIAL CELLS MAY NOT BE NECESSARY IN GASTRIC ACID SECRETION

Horace W. Davenport, Ph.D., of the Department of Physiology at the University of Michigan, Ann Arbor, Michigan, has undertaken

studies of acid secretion in an effort to discover its locus. His particular interest is in the function of the surface epithelial cells. He has reported in Gastroenterology that these cells apparently are not necessary for gastric acid secretion. The work was supported by a grant from the Division of General Medical Sciences.

The role of the mammalian gastric mucosa in acid secretion has not been unequivocally established in prior research, though it is generally agreed that parietal cells are associated in

some way with the process. Some scientists have thought it possible that cooperation between parietal cells and surface epithelial cells was necessary for secretion.

The purpose of Davenport's work was to determine whether a mucosa stripped of surface epithelial cells is still able to secrete. Using white mice five to six weeks old, the investigator pressed the stomachs of the mice against dry filter paper, thus stripping them of as many epithelial cells as possible. No stomach could be completely stripped of these cells, but most of the denuded stomachs had no more than three percent of their surface epithelial cells remaining.

Acid, in addition to lactic and carbonic acids, accumulated on the mucosal side of the denuded stomachs. Within the limits to which surface epithelial cells were removed, then, the investigator concluded that mouse stomachs do not require the presence of the cells for gastric acid secretion.

NEW FINDINGS REPORTED ON HISTOBIOLOGICAL RELATIONSHIPS IN SKIN

Three papers have reported findings bearing on several aspects of the nature of skin disorders, particularly problems of hyperpigmentation and depigmentation. The research was supported by a grant from the Division of General Medical Sciences.

The epidermis, which is the most superficial layer of skin, is composed of several cell types which in the past have been considered to be related to or derived from the same type of cell. Hermann Pinkus, of Wayne State University College of Medicine, Detroit, Michigan, reporting results of his work in an article in Pigment Cell Biology, has supported the theory that melanocytes are specific cells of neuroectodermal origin which exist symbiotically with Malpighian cells, which are of lateral ectodermal origin.

Interaction between the symbionts may be modified and disturbed in a variety of experimental and pathologic conditions. Some examples of these are various diseases of excessive or deficient pigmentation, the tumors of the skin, and various inflammatory diseases or conditions of the skin. More knowledge of the origin of the epidermal cells and their relationships to each other could play an important role in understanding pathogenesis of these diseases and developing more effective therapy.

The studies have also shown that melanocytes exist in a relatively constant number. Variations in skin color, the investigator concludes, are therefore due to the amount of pigment produced by these cells and to the quantity of pigment granules thence passed on to the Malpighian cells.

The investigator believes that though there is evidence for a symbiotic relationship between melanocytes and Malpighian cells, there is no genealogic relationship between the two. During forced regeneration, Malpighian cells reproduced rapidly while melanocytes remained passive. If the two cells had stemmed from a common, immediate precursor, one would expect the two types to appear in a relatively constant numerical relationship in this instance.

Another phase of the study has shown that under certain conditions, melanocytes can ascend into higher epidermal layers where they become high level branched cells, also known as Langerhans cells. The hypothesis that cells of Langerhans are effete melanocytes is supported in a paper by Dr. Julin Fan who has presented evidence in the Journal of Investigative Dermatology to show that the melanocyte produces pigment granules while at the basal layer of the epidermis. When it becomes inactive it is found in the more superficial layers of the epidermis and becomes aureophilic, that is, develops an affinity for gold stains. Among his studies, one, involving stimulation of the melanocytes with thorium-x, resulted in additional numbers of decreasingly active melanocytes appearing in the superficial layers of the skin. The number of Langerhans cells decreased. This along with his other studies indicates that as the melanocyte becomes inactive it becomes more aureophilic and that the Langerhans cell can therefore be interpreted as an effete melanocyte.

Dr. Pinkus presented a review of the recent studies pertaining to symbiosis in Dermatologica. He supports the theory that human epidermis is a symbiosis of several cell types analogous to the symbiosis existing in the lichens of the plant world.

CLINICAL

PULMONARY EMPHYSEMA, FIBROCYSTIC DISEASE RELATIONSHIP STUDIED

Dr. John A. Wood, of the Columbia University College of Physicians and Surgeons, New York, has reported on a study of the rela-

tionship of chronic obstructive pulmonary emphysema and fibrocystic disease of the pancreas. His findings appear in the New England Journal of Medicine. The work was supported by a grant from the Division of General Medical Sciences.

The purpose of the study was to investigate whether some cases of chronic obstructive pulmonary emphysema in adults could possibly represent atypical or aborted forms of fibrocystic disease of the pancreas. Former observations have suggested that victims of the two diseases occasionally have in common respiratory disturbances and abnormalities in the composition of sweat.

Dr. Wood observed three groups of patients. Members of one group had chronic obstructive pulmonary emphysema, members of another had fibrocystic disease of the pancreas, and those in the last were parents of children with fibrocystic disease.

Three broad groups of methods were applied: a pulmonary-function test, the sweat test, and a pancreatic-function test. The tests were aimed at establishing the concentration of chloride in sweat and the intestinal absorption of neutral fat and fatty acid. Control subjects were used to establish a baseline for data and information.

It was found that five of the 24 patients with emphysema had abnormally high concentrations of chloride in sweat, and four of these also had impaired absorption of neutral fat. During severe salt restriction, the fibrocystic subjects lost large amounts of salt in their sweat but both the parents of patients with fibrocystic disease and the emphysematous patients were able to conserve salt.

Dr. Wood's study is in agreement with previous observations of several investigators, one of whom (Dr. Paul A. di Sant' Agnese) found that some parents of fibrocystic children had pulmonary emphysema and abnormally high concentrations of sweat chloride.

The present study offers another type of support for a pathogenetic relation between fibrocystic disease and chronic obstructive pulmonary emphysema.

TECHNIQUE FOR MEASURING ESOPHAGUS MOTILITY IS DEVELOPED

James H. Pert, of the Departments of Medicine and Pediatrics, the New York Hospital-Cornell Medical Center, has described a

new technique for studying the motility of the esophagus. The work was supported by a grant from the Division of General Medical Sciences, NIH, and is reported in the Journal of Clinical Investigations.

The procedure employs electromanometers, a constant infusion pump and a specially prepared set of six catheters connected to three 3-way stopcocks. The investigators made 15 observations on ten healthy adult subjects.

The technique is described as successful in permitting sensitive, simultaneous recordings from closely approximated sites and as being sufficiently comfortable to afford prolonged observations.

Among his findings, the researcher reports the following:

"The pattern of pressure changes obtained from the vestibule is different from that of the body of the esophagus both at rest and during deglutition, indicating that each of these is a separate functioning segment.

"These motor patterns at rest and during swallowing indicate that the vestibule behaves as an intrinsic sphincter and should be referred to as the esophago-gastric sphincter."

Comparisons between normal patterns and those with disease states and during drug administration are in progress.

TRANSISTORIZED AMPLIFIER
MEASURES HEART RATE
UP TO 24 HOURS

Dr. Donald A. Rowley, of the
Department of Pathology at the
University of Chicago School of
Medicine, Chicago, Illinois,

has devised a heart beat counter which can be operated for as long as 24 hours. Dr. Rowley holds a five-year NIH Senior Research Fellowship from the Division of General Medical Sciences. The development is reported in Science.

The small, self-contained counter was developed in cooperation with engineers of the Illinois Bell Telephone Company. It weighs 100 grams and is a single unit with an amplifier, power source, and cumulative counter improvised from a watch movement. Up to 216,000 heart beats, occurring at rates from less than 40 to more than 150 per minute, can be recorded on it.

Other equipment for counting the pulse is more bulky, and activity of the individual is limited. With the new small pulse counter strapped to the chest, a person's heart beat can be measured while he sits, walks, exercises vigorously, lies in various positions, and sleeps. Factors of age, sex, and occupation are being considered in relation to the pulse count, with a view towards accurate and additional information about the effect of various activities on the heart.

TECHNIQUE DESCRIBED FOR
CRICOTHYROID BRONCHOGRAPHY
WITH POLYETHYLENE CATHETER

Dr. James K. V. Willson, of the
Department of Radiology at Johns
Hopkins University in Baltimore,
Maryland, has reported on a new

technique of bronchography which makes use of a polyethylene catheter inserted through the cricothyroid notch. His findings appear in the American Journal of Roentgenology, Radium Therapy and Nuclear Medicine. The work was supported by a grant from the Division of General Medical Sciences.

Bronchography is the roentgen-ray examination of the bronchial tree after the injection of a contrast fluid.

In the injection of the radio-opaque fluid into the bronchial tubes, two techniques have been traditionally used: the first is the tracheal catheterization method, the second the direct needle puncture of the cricothyroid membrane. Both methods have disadvantages. The former is likely to induce coughing and irritation of the larynx. The latter, due to the rigidity of the needle, restrains the movement of the patient, which is necessary for satisfactory filling of both sides of the lungs.

Dr. Willson has developed a modification of the second method. He performed 25 bronchographies and reported excellent technical results achieved without complication.

Dr. Willson's method involves the introduction of a polyethylene tube inside the needle that is used for puncture, extension of the tube three inches into the trachea, and subsequent withdrawal of the needle. With the needle removed, it is possible to move the patient easily while the lobes are being filled. The procedure is accomplished with a local anesthetic.

In comparing his method with the tracheal catheterization method, Dr. Willson points out that both should be used, the choice depending on the individual case. However, when tracheal catheterization is difficult, his method, the cricothyroid puncture with insertion of a polyethylene tube, has proved safe and easy to use.

**FETAL ELECTROCARDIOGRAM
USEFUL IN DIAGNOSING
MULTIPLE PREGNANCY**

Dr. Saul David Larks, Ph.D.,
from the Department of Bio-
physics and the Department of
Obstetrics and Gynecology,

University of California at Los Angeles, California, has demonstrated the diagnosis of a multiple pregnancy at 16 weeks using fetal electrocardiographic techniques, which he is studying. Whereas there is agreement that x-irradiation of the mother and fetus should be avoided when possible, the electrocardiographic technique can be safely used repeatedly. A report of Dr. Larks' work appears in the American Journal of Obstetrics and Gynecology. His work was supported by a grant from the Division of General Medical Sciences.

Electrocardiographic equipment with sensitivity in the micro-volt range was necessary for the tests. Eleven cases of multiple pregnancy were studied, including nine twin pregnancies, one triple, and one quadruple pregnancy. In each case, accurate diagnosis was made. Dr. Larks had reported previously that the fetal electrocardiographic impulse generally becomes readily recordable by the 19th to the 21st week of pregnancy. Occasionally the impulse appears earlier, as in the case of one of the twin pregnancies which was detectable at 16 weeks.

The investigator was also able to estimate fairly accurately the type of fetal presentation which would occur, the tracings clearly differentiating between the vertex and breech presentations at the time of the recording.

AMINOACIDURIA STUDIED
AS GUIDE TO CELLULAR
FUNCTION OF THE KIDNEY

Dr. J. Julian Chisolm, Jr.,
M. D., from the Department of
Pediatrics, Johns Hopkins
University School of Medicine,

Harriet Lane Home of the Johns Hopkins Hospital, and the Pediatric Division of the Baltimore City Hospital, Baltimore, Maryland, has investigated generalized renal aminoaciduria, finding it clinically significant in the diagnosis of certain diseases and in the further understanding of kidney function. A report of his work appears in a review article in The Journal of Pediatrics. The work was supported by a grant from the Division of General Medical Sciences.

Excessive excretion of amino acids in the urine, hyperaminoaciduria, is a sign of faulty cellular function. Although Dr. Chisolm focused his attention on impaired renal tubular reabsorption, known to be an immediate cause of generalized renal aminoaciduria, his studies also yielded considerable information on other causes of this condition related to diseases in other organs of the body.

In the past, the evaluation of amino acid studies has been limited by the lack of practical analytic methods for determining the concentrations of individual acids in a sample. Dr. Chisolm used paper chromatography as a technique capable of delineating the qualitative and semiquantitative differences in the relative concentrations of amino acids. The differences produced distinctive "patterns of aminoaciduria."

Different patterns are seen in a variety of syndromes, the most common being vitamin D deficiency states and the Fanconi syndrome. Study of the excretion patterns can often reveal whether a given hyperaminoaciduria is renal or extrarenal in origin, an important difference in establishing the nature of the underlying metabolic defect.

According to Dr. Chisolm, hyperaminoaciduria in itself, with the exception of cystinuria, is probably not deleterious, but is often the sign of a disorder which should be treated. The urinary amino acid excretion pattern points the way to diagnosis of the disorder and thus is of clinical value.

GASTRIC SECRETION
STUDIED FOLLOWING
PORTACAVAL SHUNTS

Dr. Soichi Kohatsu, from the Department of Surgery at the University of Chicago, Chicago, Illinois, has experimented with

dogs in an attempt to explain marked increases of gastric secretion following portacaval transpositions. A report of his study appears in The American Journal of Physiology. The work was supported by a grant from the Division of General Medical Sciences.

The investigator has confirmed and expanded the work of Drs. J. S. Clarke, J. C. Hart, and R. S. Ozeran who in 1958 reported on the mechanism of increased production of acid secretion and ulcers in patients with portacaval shunts. The present study has sought to demonstrate that the increase is due to some secretory hormone that is normally inactivated or excreted by the liver but has been shunted around it because of the portacaval transposition.

Dogs were prepared with vagus denervated Heidenhain fundic pouches, and their antrums were transplanted to the abdominal wall so as not to come into contact with food. Thus, the two normal mechanisms for gastric secretion (vagus nerve and antrum hormone) were rendered functionless. Usually such an action would result in minimal secretion, but after portacaval transposition, an abundant secretion of gastric juice occurred in response to food taking. By mechanisms which are not yet positively known, but which must be humoral in nature, the portacaval transposition brings about the secretion increase.

Earlier observations by other scientists have pointed to a secretory hormone in the intestines as the cause, but the investigator feels that because the intestinal phase has such little effect upon secretion, a nonspecific gastric secretagogue from the ingested food, inactivated by being shunted around the liver, may be responsible for the increase.

IRRADIATION EFFECTS
ON PRIMARILY CLOSED
WOUND STUDIED

Dr. Dale B. Flickinger, of the Department of Surgery, Harvard Medical School; Fifth Surgical Service, and Sears Surgical

Laboratory, Boston City Hospital, Boston, Massachusetts, has found that experimental wounds, closed immediately after exposure to sublethal total body irradiation, will in most cases heal normally. The work is reported in Surgical Forum. The research is supported by a grant from the Division of General Medical Sciences.

Three groups of young, growing rats were incised for the studies. Sponges were implanted in the subcutaneous tissue of

each incision and the wounds were then sutured primarily. The first group served as controls, those in the second group were given 200 roentgen total body irradiation, and those in the third, 600 r.

At the sixteenth day after wounding, there was no significant difference between the controls and the second group. The latter, during the first few days, showed a mild leucopenia, a slight depression of collagen formation, and a slower weight gain. In the more heavily irradiated third group, 43% died within 30 days, and there was anemia, leucopenia, and a marked suppression of weight gain, as was expected, in all animals. In spite of these severe bodily changes, however, collagen production was moderately depressed only in the first 12 days, returning to normal by the sixteenth day. Previous studies have shown that early closure of experimental lesions results in normal healing within 14 days as measured by wound strength and contracture.

The experimental data established that in granulation tissue obtained by the sponge biopsy technique from a primarily closed wound, collagen concentration became normal within 16 days in the totally irradiated rat, even in the presence of anemia, leucopenia, and weight loss.

The investigator feels that early closure of wounds is probably desirable after total body irradiation, and that normal healing may generally be expected.

ZIRCONIUM FOUND TO BE A CAUSE OF AXILLARY GRANULOMA

The demonstration that axillary granuloma may be caused by an allergic hypersensitivity to the metal zirconium is reported by

Dr. Harry J. Hurley, Jr., of the University of Pennsylvania, Philadelphia, Pennsylvania, in the Henry Ford Hospital Medical Bulletin.

He has found that zirconium-containing stick deodorants may cause the formation of granulomas in the human axilla.

According to his report, "This to our knowledge is the first demonstration in man that an allergic process can be responsible for the development of a granuloma." The discovery may have significant effects on the study of granulomatous disorders in man, and may point up the need for a review of the uses of zirconium in medicine and surgery.

OXYTOMIC AND ANALGESIC
DRUG EFFECTS STUDIED
WITH ELECTROHYSTEROGRAM

S. D. Larks, Ph.D., of the
Department of Biophysics, and
the Department of Obstetrics
and Gynecology, School of

Medicine, University of California at Los Angeles, California, has made studies of the electrical activity of the parturient uterus and has established that oxytocin may be an important factor in the maintenance of active labor. His work, supported by a grant from the Division of General Medical Sciences, was reported in Obstetrics and Gynecology.

The investigator administered a natural form of oxytocin in 23 cases; in 13 cases he gave oxytomic 202, which is not oxytocin but an oxytomic compound; and in five instances, he used a synthetic oxytocin. The rate of conduction velocity was related to the amount of material being administered. In one case of normal labor, Demerol was used as a test of analgesic effect.

Readings of the normal human uterus during labor were taken as a means of comparison with those taken after drugs had been administered.

The effects of the drugs were recorded with a new and sensitive tool, the electrohysterogram.

Both unipolar and bipolar readings were obtained. The unipolar leads consisted of an indifferent electrode on the thigh paired with an exploring electrode over the uterus, and the bipolar consisted of electrodes over the right or left side of the uterus.

It was found that the mechanism of action in the uterus is influenced by the drugs. With the natural and synthetic forms of oxytocin, there was a decided increase in the rate of conduction of impulse in uterine muscle, but the pacemaker was not affected. Oxytomic 202 had an effect both on the pacemaker and the conduction of impulse. From the tracings it was established that synthetic oxytocin produced the same effect as natural oxytocin. Whether or not oxytocin has anything to do with the onset of labor is not yet clear.

A transient slowing of labor is produced by Demerol. Electrical analysis suggests that this slowing may be a result of interference with conduction, and secondarily a result of some change in pacemaker timing.

Among other benefits, the work has demonstrated how the electrohysterogram can be used in studying the physiologic and pharmacologic aspects of the uterus.

ENVIRONMENTAL HEALTH

SAMPLER DEVELOPED
TO MEASURE GASEOUS ACID
IN DOMESTIC PREMISES

J. J. Phair, of the Department of Preventive Medicine and Industrial Health, University of Cincinnati College of Medicine,

Cincinnati, Ohio, has reported in the British Journal of Industrial Medicine on an air sampler for the estimation of gaseous acid in domestic premises. His work was supported in part by an NIH research grant.

The sampler, which is small enough to be carried easily by one man, gives six-hourly readings. With special methods for minimizing water loss, the sampler can provide readings on the amount of sulphur dioxide in a suburban atmosphere and an efficiency of 95 percent can be obtained with a single-stage sampling.

During most periods of high atmospheric pollution, gaseous acids, particularly sulphur dioxide, reached higher levels, often causing pulmonary disturbances among persons with a small respiratory reserve, and forcing them to retire to their beds. The air sampler will aid researchers in their studies of pollution factors within the microenvironment of the home, and aid in the prevention or treatment of disorders caused by such pollution.

FINDINGS PUBLISHED
ON CASE STUDIES
OF TRAFFIC ACCIDENTS

J. Stannard Baker, of the Traffic Institute of Northwestern University, has published findings after the first year of a three-

year project in traffic accidents. The work is being supported by the U. S. Bureau of Public Roads and by grants from the Division of General Medical Sciences.

The investigative method involves an inter-disciplinary approach, in which a physician, a psychologist and an engineer study accidents within minutes after they occur.

"The psychologist and physician examine the vehicle," according to the report, "observe the road situation and . . . interview the persons involved The engineer makes observations of the road situation and vehicle at the scene and conducts post-accident investigations. A consolidated report is made . . . in each case."

During the first year, 157 traffic accidents were investigated, and 32 of these were followed with intensive post-accident interviews. All the accidents investigated occurred in Evanston, Illinois.

Tentative case findings suggest that the following were significant factors in the accidents investigated: 1. Deficiencies in road design. 2. Obstructions to the driver's view. 3. Interactions of a social nature within vehicles. 4. Deficiencies in the driver's knowledge. 5. Inaccuracies in predicting the actions of others.

The researcher found that scientists are able to get to the scenes of accidents quickly, under the conditions necessary for research, and that drivers will cooperate in interviews, particularly at the request of the police.

Findings included the fact that road deficiencies and social forces were more commonly associated with accidents than had been expected, and that the accidents seemed frequently to involve normal people doing usual things.

Future work in the project will include efforts to develop measuring instruments for better assessing accident factors.

INSTRUMENT DEVELOPED FOR THE ANALYSIS OF AIR-BORNE PARTICLES

Dr. Alexander Goetz, of the California Institute of Technology in Pasadena, California, has reported on the aerosol

spectrometer, a new instrument capable of analyzing air-borne particles in the submicron range. His findings appear in the Public Works Magazine. The development work was supported by a grant from the Division of General Medical Sciences.

Aerosols are those air-borne particles which, unlike dusts and most smokes, do not settle out but remain suspended for long periods of time. In recent years, there has been interest in them because of the role they play in the complex problems of air pollution. Their physical and chemical interaction with gases in the atmosphere increases the irritating and damaging effects of these gases. Since the aerosols are extremely small, they are difficult to work with and, consequently, knowledge about them is limited. A program has been underway since 1955 at the California Institute of Technology to develop methods of precipitation and concentration of natural and artificial aerosols in the submicron range. From this effort has come the instrument which Dr. Goetz describes.

The working principle of the aerosol spectrometer is based upon the application of a large centrifugal field to a continuous flow of the gaseous suspension through helical channels around the outside of the rotor. The design of the machine is an improvement over other attempts because it lessens the shear forces which act on the particles. With this machine, the particles are not disrupted or torn apart in the separation.

The spectrometer allows investigators to work closely with the aerosols, and to make direct measurements of both their physical and chemical properties. It eliminates the danger of the small aerosols being obscured by the larger sizes and allows for the testing of the outflowing air from which the particles have been removed. For example, it was shown in a particular set of tests that eye irritation caused by auto exhaust that has been exposed to sunlight or its equivalent could be largely eliminated by the precipitation of particle sizes down to 0.15 microns; plant damage, however, did not diminish. In time, also, the instrument should lead to a more exact evaluation of the performance of air filters, precipitators, and similar devices.

The aerosol spectrometer has recently become generally available as a standardized model.

TOXICITY INCREASES FOUND IN COMBINATIONS OF INSECTICIDES

Kenneth P. Dubois, of the Department of Pharmacology, University of Chicago, Chicago, Illinois, in an article in the A.M.A.

Archives of Industrial Health, has revealed that in 3 of 15 combinations of certain commercially-produced insecticides there were ten to 50-fold increases in toxicity. This poses a possible hazard for both manufacturing workers and consumers, and suggests that related toxic reactions may be irregular and not predictable.

In his paper, Dr. Dubois refers to earlier work by Dr. J. P. Frawley of the Food and Drug Administration in determining that potentiation of toxicity results from the simultaneous administration of ethyl paranitrophenylthiobenzenephosphonate (EPN) and malathion and to similar work by J. A. Rider using octamethyl pyrophosphoramidate (OMPA) and neostigmine.

Dr. Dubois has carried the work farther with 15 tests using nine organic phosphates which have been approved for use on food crops. These include parathion, methyl parathion, demeton (Systox), EPN, malathion, guthion, phosdrin, diazinon and triethion.

In six tests, the effects were simply additive. In six other tests, the effects were less than additive, while in three, the effects were potentiated with ten to 50-fold increases in mortality to the subject animals.

In his summary, Dr. Dubois writes: "Our present knowledge of the problem of potentiation of the toxicity of organic phosphates does not provide an answer to the question of whether or not this effect constitutes a health hazard in connection with

consumption of contaminated food. It is clear, however, that acute exposures to certain combinations of organic phosphates on the same or successive days during the manufacture or use of these materials should be avoided. The further screening of compounds by acute toxicity tests followed by subacute tests should indicate whether there is any health hazard at the proposed safe levels for new compounds, assuming that the susceptibility of man resembles that of the experimental animals upon which the tests are conducted. Studies on the mechanism responsible for potentiation of the toxicity of one organic phosphate by another agent of the same class have indicated that the effect occurs when one compound interferes with the enzymatic detoxification of another compound by inhibiting an esterase in liver, serum, and other tissues. The low mammalian toxicity of some organic phosphates, such as malathion and 1,1,-dimethyl, 1-hydroxy-2,2,2-trichloroethylphosphonate (Dipterex), is highly dependent upon efficient detoxification. Interference with the detoxification of these compounds markedly increases the mammalian toxicity. To date consideration has been given only to inhibition of the detoxifying esterase by organic phosphates . . .

"Aside from practical considerations, the finding of agents capable of inhibiting hydrolytic detoxification reactions constitutes an important advance from the standpoint of possible usefulness in basic research. Enzyme inhibitors have long been profitably employed as tools in the study of normal metabolism, and it seems likely that agents like EPN, Dipterex, and other potentiating compounds can be employed profitably for this purpose in the future."

LAW STUDY COMPLETED ON HOSPITAL OPERATIONS

The Health Law Center of the Graduate School of Public Health, University of Pittsburgh, Pitts-

burgh, Pennsylvania, has completed a two-year, detailed study of state laws as they affect the operations of hospitals. The investigator was John R. McGibony, M.D., Professor of Medical and Hospital Administration. He and his staffs visited 31 states in the course of their research and carried out a pilot study of the legal problems of 200 hospitals in one state.

A two-volume manual has been published. One volume is for administrators, designed for quick reference when problems of legal import occur in the hospital. Each of the chapters, arranged alphabetically, has a table of contents to help pinpoint the area of interest and a cross-reference guide aids in finding related materials. Charts indicate the pertinent laws in each state.

The second volume is for attorneys. It is organized similarly to the administrators' volume, but with each chapter containing a discussion of specific legal points with emphasis on the administrative and legal considerations of a hospital. Contrasting lines of legal authority and various types of statutes are analyzed and compared, cogent judicial reasoning is quoted, and recommended procedures, with model hospital forms, are included. More than 3,000 judicial decisions are analyzed or cited.

The volumes, in loose leaf form, are available on a three-year subscription for \$150.00. This includes a service which every quarter will provide new text material, charts and forms reflecting changes in the law, new developments in the field of hospital administration and further research as part of the Health Law Center's continuing program.

The study, thought to be the first of its type, is considered a significant contribution to hospital administration because of the increasing amount of legislation and regulation in the field and variations from state to state.

AVAILABLE KNOWLEDGE
ON EXPOSURES TO
BERYLLIUM SUMMARIZED

Harriet L. Hardy, M. D., of the Occupational Medical Clinic, Massachusetts General Hospital, Boston, Massachusetts, has emphasized the need for physicians to understand the available

knowledge of intoxication following exposure to toxic beryllium compounds in harmful amounts. As reasons for this need, she points out the delay -- often 5 years -- between the last known exposure to beryllium and the onset of the disease; and the increasing use of the metal in industrial manufacturing. In 1958, 601 cases of beryllium poisoning were recorded and new cases continue to be reported.

(The Washington Post of June 12, 1959 quotes The Martin Co., Baltimore, as announcing a "major breakthrough" in fabricating beryllium sheet for use in missile construction, with many advantages in this use over other structural materials. This would indicate additional increases in the production and use of beryllium.)

The review by Dr. Hardy in the Journal of Chronic Diseases was supported by a grant from the Division of General Medical Sciences.

The earliest papers on the disease were written in 1933 in Germany, followed shortly by reports from Italy and Russia. These early interests were occasioned by the exposures connected with the extraction of beryllium from the ore, and it therefore was felt until recently that the extraction of

beryllium, and the manufacture of fluorescent lighting, were the main causes of beryllium poisoning.

Dr. Hardy reports that the list of sources now, however, has been lengthened to include neon sign manufacturing, ceramic operations, radio tube manufacturing, atomic energy development, the use of alloys containing more than 4 percent beryllium and the contamination in neighborhoods in the vicinity of manufacturing operations.

Of 40-odd instances of beryllium poisoning in a neighborhood, more than half of the patients had been exposed to fine dust from workers' clothes, brought home from factories where beryllium had not been thought harmful. Of the remaining cases, all but a small number lived near beryllium-using plants which took no precautions against air contamination.

Present evidence indicates a variation in toxicity of beryllium compounds, depending upon physical or chemical characteristics not now defined.

In a review of more than 300 cases, Dr. Hardy found that 41 percent of the cases began within a month following the last exposure. Twenty-nine percent did not appear for one to 5 years following the last known exposure, and 18 percent did not appear for 5 to 10 years.

Acute cases of the disease are characterized by dermatitis, and possibly conjunctivitis and/or irritation of part or all of the respiratory tract.

A few patients with acute poisoning apparently recover and later develop a chronic disease. The majority of chronic cases, however, do not appear for several weeks or several years. Pneumonitis is the most striking clinical feature of the chronic disease, but skin nodules and renal stones have been reported. Skin granulomas appear spontaneously in a few patients no longer exposed to beryllium. On biopsy, beryllium is found in the tissues. It can also be found in the urine. The most common symptoms are dyspnea on exertion and cough, usually nonproductive. Weight loss, with or without anorexia, weakness and fatigue are frequent.

Prevention can be very effective through the better education of manufacturers using beryllium and beryllium compounds.

"There is considerable speculation as to the mechanism of production of detectable disease following . . . exposure," Dr. Hardy wrote. "Experimental animal studies . . . have produced inconsistent results. Granulomatous lesions of lung, liver and subcutaneous tissue have been reported. Lung malignancy has

appeared in significant numbers in experiments with at least one laboratory animal exposed Thus far, there has been no evidence of beryllium-induced malignant disease in humans. However, since the clinical picture is still unfolding, it will be some time before all the possibilities of toxic beryllium have been detected and accurately recorded."

The most successful treatment today is with the steroids. Intensive treatment with ACTH produces both subjective improvement of the patient and objective change in abnormal cardio-respiratory function.

Dr. Hardy wrote that despite the success of the steroids it is not yet possible "to speak of a cure of berylliumosis" through their use.

HIGHLIGHTS OF PROGRESS

IN SUPPORT OF RESEARCH

AT NIH

1959

Items of Interest on Research Studies and Program Developments in the Division of Research Services

Today's medical scientist, in order to make maximum progress, works closely with teams of highly skilled professional and technical personnel, trained and equipped to provide a variety of technical, scientific, and engineering services. These services are provided daily by the Division of Research Services for scientific personnel at NIH and for the Institutes as a whole.

The Division operates an animal hospital, animal receiving center, and animal farm for the use of research investigators; it breeds laboratory animals and provides technologic services for the production of germfree animals. It serves NIH research needs through a variety of other necessary services—for example, by providing clean glassware and bacteriological and tissue culture media, by administering a 75,000-volume medical library, and by designing exhibits for national and international scientific meetings. Biometric consultation, computation and data processing, and technical illustrating, translation, photographic, and publication services are also available through the Division's service units. In addition, the Division develops plans for the location, construction, remodeling, and maintenance of all NIH buildings and laboratories. Much of this work finds its way into the literature in the form of guides, texts, and manuals on laboratory design.

The more than 800 employees who perform these services include veterinarians, pathologists, bacteriologists, geneticists, biologists, and horticulturalists; engineers, architects, mathematicians, and draftsmen; instrument-makers, machinists, and glassblowers; as well as many other specialists who perform a variety of essential, productive, and creative tasks.

DRS scientists and technologists often collaborate with Institute investigators on problems that encompass both the research under way and the service aspects of that research. Precision instruments and other highly complex research equipment not available

commercially are designed, developed, or modified to meet specific and immediate research needs. More than 50 percent of all instruments fabricated are in this category of non-standard design.

To maintain the high quality of its many services, the Division anticipates the needs of those conducting the research and initiates studies relating specifically to the services provided. Methods of equipment sterilization, developments in animal surgery techniques, and ventilation studies aimed at reducing the volume of airborne contaminants in research areas are among the studies undertaken.

Cooperative or individual research conducted by DRS personnel has a direct application to the NIH research program and often results in publication in technical or professional journals and these research developments are thus shared with other medical institutions or with commercial manufacturers.

MULTIPLE SYRINGE DRIVE SPEEDS BACTERIOPHAGE TYPING

Automation in the laboratory is speeding up many processes usually performed by hand.

An example is an idea for the mechanization of bacteriophage typing in the Clinical Pathology Department of the Clinical Center. By means of a repeating multiple syringe drive, which was developed by the Instrument Section, Laboratory Aids Branch, it is possible to complete in less than one hour phage-typing tests for staphylococcus which formerly took 10 hours.

With the new mechanism, 26 glass syringes suspended from a disk can be filled simultaneously with phage held in suspension in a nylon cup block. The syringes are filled, emptied, and cleaned without disassembly; the entire apparatus, which is of plastic and stainless steel, can be sterilized before and after use. Phage suspensions are drawn into the syringes by the action of individual plungers and released in droplets by the turn of a calibrated screw via plastic-sleeved needles onto petri dishes or plastic plates filled with solidified agar.

The syringes will apply phage to 160 plates before refilling is necessary; phage may be applied to as many as 300 plates an hour.

The multiple syringe drive has many potential laboratory uses: for testing antibiotic sensitivity; for the transfer of a variety of bacteria cultures; or for replica plating in the selection of bacterial mutants in genetic studies.

INTENSIVE STUDY OF PRIMATE DISEASES LAUNCHED

The Comparative Pathology
Section of the Laboratory
Aids Branch is engaged in a

collaborative study to collect and report data on etiologic and environmental factors that may cause illness in rhesus monkeys imported from India for medical research.

Sponsored by the National Advisory Committee on Primates, the study is being carried out cooperatively with scientists at Christ Hospital Institute for Medical Research, Cincinnati, and Parke-Davis and Company, Detroit. Also participating are consultants from the Leptospira Research Laboratory in the Communicable Disease Center of the Public Health Service and from the University of Pittsburgh and Wayne University Schools of Medicine.

Each of the three collaborating groups is studying 200 monkeys; 100 of the animals are receiving treatment for diseases contracted during and after their journey to this country, and 100 are being studied and tested in a variety of ways as untreated controls. The initial phase of the investigation began with the tattooing (for identification) of the test monkeys in India and the collection of serum specimens for antibody studies and of fecal specimens for viral and bacterial isolation studies.

The improvement of sanitation and therapeutic measures, following the capture of the monkeys in India and continuing during transit to the United States, is expected to reduce the incidence and severity of illnesses encountered. The corresponding reduction in mortality anticipated would thereby increase the supply of monkeys for research.

HIGH-VOLTAGE POWER UNIT DESIGNED FOR ELECTROPHORESIS APPLICATION

A 5,000-volt power unit is
being constructed by the
Instrument Section, Laboratory

Aids Branch, for use in high-voltage electrophoresis in the Institute of Arthritis and Metabolic Diseases and in the Heart and Mental Health Institutes.

AUTOMATIC DEVICE CONTROLS BLOOD VOLUME DELIVERED BY HEART PUMP

Successful cardiopulmonary
bypass requires that the
blood level in the heart

oxygenator be kept constant under varying conditions of arterial and venous flow. The Instrument Section, Laboratory Aids Branch, has devised for this purpose an instrument that automatically detects the rise and fall of blood and maintains synchronous speed in a reversible rotating pump attached to a blood reservoir. Blood level in the oxygenator, a component of the heart-lung pump, accurately reflects the volume of blood within a patient undergoing heart surgery.

The automatic system consists of four platinum electrodes embedded in silicon rubber; it is also connected to an electromagnetic flowmeter and an electronic circuit. A timing device eliminates shunting of the motor when the blood level is adjusted.

One electrode provides the ground return for the other three. As the blood level in the oxygenator changes, the second and fourth electrodes energize the reservoir pump, causing it to rotate in the proper direction. The third electrode stops the reservoir pump as soon as balanced conditions exist. As a further safety device, the heart-lung pump automatically stops when the blood level in the oxygenator falls below the level-sensing electrodes, thus preventing the injection of air into the patient's bloodstream. The system is in routine clinical use, operating automatically, semi-automatically, and manually.

NIH HOLDS OPTION TO BUY NEW ANIMAL FARM

The Laboratory Aids Branch maintains temporary, local farm facilities for housing

large animals that are subjects of long-term research studies. Plans are now nearing completion which will provide more permanent and adequate facilities for this purpose elsewhere.

Late in 1959 an option was secured to purchase a 500-acre site in a rural area of northwestern Montgomery County, 25 miles away from NIH. The site is near enough to NIH to provide easy access, yet sufficiently remote to protect its future use as a research facility. If the transaction is completed, the site will be transformed into a permanent animal farm, where horses, cows, swine, sheep, goats, and other large animals will be safely housed, fed, and pastured.

Here the Division of Research Services will install modern water supply and waste disposal systems and will construct the necessary animal buildings. Following receipt of a topographical engineering survey and recommendations from the Institutes, a master site plan will be prepared showing the location of buildings, roadways, power lines, and other utilities. Each Institute will use the research facilities of the permanent farm much as it does the Clinical Center, and the Division of Research Services will have the responsibility for operation and maintenance. Several of the Institutes have already made far-reaching plans for future use of the farm.

SATISFACTORY METHOD FOUND FOR COATING MICROELECTRODE TIPS

The Instrument Section, Laboratory Aids Branch, has succeeded in developing a

coating to prevent polarization on platinum electrode tips, a major difficulty when extended electrolytic tests are performed. Electrolysis is widely used in biomedical research, and polarization often develops.

By experimentally interchanging three variables, platinum electrode tips (5 mm. by 1 mm.) joined to stainless steel wire were coated electrolytically with platinic chloride. The three variables were the concentration of platinic chloride, the magnitude of the electrolytic current, and the length of immersion time.

The coating method makes possible uninterrupted electrolytic experiments of long duration. The Surgery Branch of the National Heart Institute uses coated electrodes to detect the amount of hydrogen concentrate in blood. Success with the blood studies depends on a constant flow of electrolytic current, undiminished by polarization. It has not been necessary to interrupt the 45-minute test runs to clean electrodes or to recoat them between tests.

LINE-OPERATED POWER SUPPLY ELIMINATES VOLTAGE "DRIFT"

A movable anode tube converts muscle movements into electric signals as the anode is moved

by the muscle. It thus serves as a transducer and at the same time acts as an amplifier.

In using a movable anode transducer tube for measuring tension changes within muscle fiber, investigators in the National Institute of Arthritis and Metabolic Diseases found that they could not rely on standard electric batteries. The voltage supplied is subject to fluctuation, and this is a cause of drift in direct-current amplifiers.

To eliminate the problem, the Instrument Section of the Laboratory Aids Branch has developed a line-operated, transistorized system aimed at controlling the variations. The system is small, can be attached to any 110-volt convenience outlet, and lends itself to ready use in any laboratory.

A bridge circuit connects tube and variable resistors in such a manner as to reduce 20-volt variations to 2 millivolts. A transistorized voltage regulator circuit provides constant voltage to the filament. Substantially drift-free, the system regulates the power supply and eliminates variations in voltage output to permit reliable tensiometer readings. Construction details of the system have been published in the Journal of Applied Physiology by George I. Johnston and Gerald G. Vurek.

QUALITY CONTROL TECHNIQUE ASSURES GENETIC HOMOGENEITY OF INBRED MICE

A rapid skin-grafting technique has been devised to check on the genetic purity

of inbred mice. Once the use of the technique becomes an established routine in the Laboratory Aids Branch, stringent quality control can be maintained.

Skin-grafting is an extremely sensitive procedure for determining the genetic homogeneity of scientifically mated mice. It is normally successful only between animals with very closely related genetic constitutions and has been used on occasion to differentiate between fraternal and identical twins in cattle. The usual skin-grafting procedures are laborious and time-consuming, especially when used on a large scale as in the NIH program, where the genetic quality of pedigreed mice must be guaranteed before release to researchers.

The new method is a simple technique for transferring slices of tail-skin from production mice to pedigreed hosts. A tiny piece of tail-skin from the donor mouse is grafted onto the tail of the host mouse. Shaving is not necessary. To prevent the animal from gnawing at the fresh graft, a length of glass tubing is slipped over its tail and is firmly secured with a wound clip. The glass tubing eliminates the need for bandages and all other dressings and has the added advantage of permitting continual observation of the grafted area.

The procedure was developed by LAB geneticists for the Animal Production Section. At present, the geneticists are controlling the production of the inbred nucleus of 14 strains of inbred mice, 9 strains of inbred rats, and 2 strains of inbred guinea pigs.

GAS STERILANT COULD SUPPLEMENT AUTOCLAVING OF HOSPITAL ITEMS

Investigations by the
Sanitary Engineering Branch
are of direct benefit to

hospitals and laboratories, where traditional sterilization techniques can damage heat-sensitive equipment. Many surgical instruments contain inaccessible parts that are difficult to sterilize.

NIH field studies have evaluated the mechanical and bacteriological performance of a commercially manufactured sterilizer using a low-temperature gas sterilant. A variety of materials and scientific apparatus, together with representative test organisms were exposed to a wide range of conditions. The gas is a non-flammable mixture of Freons and ethylene oxide. The active agent, ethylene oxide, has an extremely lethal effect on bacteria, spores, and fungi. The mixture promises to be highly satisfactory if recommended safety and test precautions are taken.

Surgical rubber gloves were in nearly new condition when run through six different gas-sterilization cycles for a total of 17 hours. A cystoscope and a pharyngoscope sterilized under similar conditions showed no signs of corrosion; the lenses remained clear and the cementing compound did not weaken. A sufficient

concentration of gas reached the inner surfaces of X-ray catheters to kill the spores of Bacillus globigii, a highly resistant organism.

As a result, gas-sterilization methods and equipment are now routinely used at NIH for sterilization of plastic components of the heart-lung machine, heart catheters, infected oxygen tents and books, and other heat-sensitive materials.

RADIATION STERILIZATION OF GERMFREE ANIMAL DIETS

Irradiation is proving to be a practical method for sterilizing the diets of

germfree guinea pigs. A satisfactory method has been developed through the collaboration of the germfree services of the Sanitary Engineering Branch and the Laboratory of Germfree Animal Research, National Institute of Allergy and Infectious Diseases. Irradiation is accomplished on the 3-million electron-volt Van de Graaf accelerator operated by the Radiation Branch of the National Cancer Institute.

Difficulties encountered in providing a steam-sterilized diet that will not retard the normal growth and development of germfree guinea pigs led to the successful use of irradiation for diet sterilization. During evaluation studies, more than a half ton of diet materials was sterilized for germfree guinea pigs and normal control animals.

SUCCESSFUL METHOD OF LENGTHENING INJURY-SHORTENED WEIGHT- BEARING BONES

The prosthetic replacement of segments of living tissue presents a challenge to modern surgery since there

is no perfect substitute for the living structure. In attacking this common problem, veterinary surgeons in the NIH Animal Hospital, Laboratory Aids Branch, have had considerable success in restoring to their original length, weight-bearing bones in the dog which have been shortened as a result of injury.

A stainless steel prosthesis is inserted into the gap formed by the removal of damaged bone and is held in place by a stainless steel pin running lengthwise through the prosthesis and extending in both directions into the medullary canal of the opposing bone shafts. Four-sided points at both ends of the prosthesis help prevent rotation.

Stainless steel, because of its greater rigidity, replaces titanium, a lighter metal used in an earlier series of operations where the weight of the animal was not so great a factor. The tightly fitting prosthesis enables dogs to be ambulatory during the recovery period and does not require external splinting. The surgical procedure is also providing information of value regarding grafting materials used to hasten bone regeneration.

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